

# Open Research Online

---

The Open University's repository of research publications and other research outputs

## Analysis of retinotectal regeneration in goldfish using polar dimensions: temporal sequence and spatial order

### Thesis

#### How to cite:

Mansfield, David Colin (1985). Analysis of retinotectal regeneration in goldfish using polar dimensions: temporal sequence and spatial order. PhD thesis. The Open University.

For guidance on citations see [FAQs](#).

© 1985 The Author

Version: Version of Record

---

Copyright and Moral Rights for the articles on this site are retained by the individual authors and/or other copyright owners. For more information on Open Research Online's data [policy](#) on reuse of materials please consult the policies page.

---

[oro.open.ac.uk](http://oro.open.ac.uk)

D 66725 /86

UNRESTRICTED

ANALYSIS OF RETINOTECTAL  
REGENERATION IN GOLDFISH  
USING POLAR DIMENSIONS:  
TEMPORAL SEQUENCE AND SPATIAL ORDER

DAVID COLIN MANSFIELD B.A.

SUBMITTED FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY  
OF THE OPEN UNIVERSITY  
DEPARTMENT OF BIOLOGY

*date of submission: March 1985*  
*date of award: 15.10.85*

MARCH 1985

ProQuest Number: 27775896

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent on the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 27775896

Published by ProQuest LLC (2020). Copyright of the Dissertation is held by the Author.

All Rights Reserved.

This work is protected against unauthorized copying under Title 17, United States Code  
Microform Edition © ProQuest LLC.

ProQuest LLC  
789 East Eisenhower Parkway  
P.O. Box 1346  
Ann Arbor, MI 48106 - 1346

VOLUME ONE

TEXT



# ABSTRACT

Quantitative analysis of electrophysiological visuotectal maps using polar dimensions demonstrated uniform representation of visual field on goldfish optic tectum, with topography equally precise in normal and "mature" regenerated projections. <sup>The precision of</sup> Circumferential topography exceeds and is not correlated with <sup>that of</sup> radial. Radial orderliness may be poor without diminution of circumferential order: these two dimensions of pattern could be generated separately from each other. The method of analysis also allows quantitation of the orientation of the retinotectal projection.

During development radial retinotopy in the sequence of axon growth could contribute to radial topography by confining optic terminals to specific annuli within the projection. The possibility of pattern formation by this means during regeneration was studied by examining the sequence of fibre growth by retrograde labelling of ganglion cells with horseradish peroxidase (HRP), applied to a cut through the optic tract at successive intervals after optic nerve transection in mid-orbit. Most results indicated a central-to-peripheral sequence of regeneration; others showed absence of retinotopic sequence.

Regeneration of axons from central retina was delayed by repetition of axotomy. Subsequent visuotectal maps were either normal or included electrophysiologically weak representation of central visual field on peripheral tectum, outside or superimposed upon the map of peripheral field. Some of the abnormal maps also contained normal representation of central field on central tectum. Because of the contradictions in these results, the influence of temporal sequence on spatial order of connections remains uncertain.

Formation of circumferential topography was investigated by anterograde tracing with HRP of axons from narrow sectors of retina in normal and regenerated pathways. Juxtaposition of labelled axons appears to be completely lost in the optic chiasm or at the site of optic nerve transection. Contact guidance of axons therefore cannot explain circumferential order in the map. Labelling was inadequate to show whether regenerating fibres resegment retinotopically in the optic tract.

## ACKNOWLEDGEMENTS

For the scientific apprenticeship which this work embodies I am deeply indebted to Dr. Nicholas Dawney, my supervisor at the Department of Anatomy, University of Cambridge: it has been my pleasure and my privilege.

I thank Dr. M. G. Stewart, my supervisor at the Open University, for encouragement and advice.

Many individuals have given generous assistance. I am particularly grateful to Professor B. Wood, Mr. R. P. Gould and Mr. M. Turmaine for access to microscopical and computing facilities at the Middlesex Hospital Medical School; to Professor O. C. J. Lippold for the loan of a word processor in University College London; and to Uma Shahani.

The writing and execution of the computer program for calculation of polar tectal coordinates is entirely the work of Mr. B. Jacobs: this patient and generous help I acknowledge with special thanks.

For one year of this work I was supported financially by the Wellcome Trust, to which body I am duly grateful.

Some of the retinae presented in chapter three have previously been described as part of my undergraduate research project, and have been published in abstract form in the Journal of Anatomy (1982).

## CONTENTS

CHAPTER	PAGE
1 INTRODUCTION	1
2 MATERIALS AND METHODS	36
3 RESULTS OF RETROGRADE LABELLING OF REGENERATING FIBRES	50
4 DISCUSSION OF RETROGRADE LABELLING OF REGENERATING FIBRES	53
5 METHOD OF ANALYSIS OF VISUOTECTAL MAPS	65
APPENDICES:	
A Evidence supporting the adoption of a spherical model of the tectum.	81
B Computation of polar coordinates "r", "c" for a spherical tectum.	83
C Computation of polar coordinates "r", "c" for a horizontal planar model tectum.	91
D Calculation of product moment correlation coefficients.	92
E Computer program (Basic) for calculation of polar coordinates for three dimensional tectum.	94
6 RESULTS AND DISCUSSION: VISUOTECTAL MAPS OF NORMAL AND REGENERATED PROJECTIONS	98
7 RESULTS: VISUOTECTAL MAPS OBTAINED AFTER INTERRUPTED REGENERATION	116
8 DISCUSSION OF VISUOTECTAL MAPS	130
9 RESULTS: ANTEROGRADE TRACING OF AXONS ORIGINATING FROM NARROW SECTORS OF RETINA	148
10 DISCUSSION OF RESULTS OF ANTEROGRADE TRACING EXPERIMENTS	156
11 CONCLUSIONS	167
BIBLIOGRAPHY	183

## INTRODUCTION

In many nervous systems there are connections between one group of neurons and another in which adjacent areas of the presynaptic set project to adjacent areas in the postsynaptic array. This may be seen in one dimension in the preservation of the tonotopic organisation of the cochlea in the auditory pathway of mammals (Merzenich and Kaas, 1980), and in two dimensions in the visual projections to the optic tecta of fish (Jacobson and Gaze, 1964) and amphibia (Gaze, 1958), and to the superior colliculus (Apter, 1945, Cooper, Daniel and Whitteridge, 1953) and striate cortex (Talbot and Marshall, 1941) in mammals. A similar pattern of organisation is also seen in the somatosensory and motor pathways (e.g. Murphy et al., 1978; Biber, Kneisley and La Vail, 1978; Dawney and Glees, 1981) and elsewhere in the central nervous system (e.g. the projection from the hippocampal to the septal area, Siegel, Edinger and Ohgami, 1974; Martin and Perry, 1983). Clearly this pattern of interconnections represents an ubiquitous and important unit of organisation in diverse nervous systems: to discover the processes responsible for this orderliness would be a major contribution to understanding the development of complex organisms.

The morphological techniques used to demonstrate the preservation of neighbourliness in the projections of adjacent groups of neurons include electrophysiological recording of selectively evoked potentials (e.g. Gaze, 1958; Maturana et al., 1959, 1960), identification of axons or their terminals by autoradiographic (e.g. Meyer, 1980; Scalia and Arrango, 1979), histochemical (e.g. Dawney, 1981b) and dye (e.g. Yezierski and Bowker, 1981; Thanos and Bonhoeffer, 1983) labelling, and the study of the functional deficit (Sperry, 1951).

and the distribution of degeneration products (Lazar, 1971; Scalia and Arrango, 1979) following selective ablation of cells or fibres. These

methods have demonstrated not only the normal arrangement of presynaptic axon terminals relative to each other but also the normal pattern of correspondence between pre- and post-synaptic sets of neurons. It may not be assumed that the same developmental mechanisms are responsible for both of these anatomical results: the orderliness within the array of presynaptic axon terminals could originate in a manner which contributes nothing to the correct placement of that population of axon terminals on their postsynaptic target. Indeed, all of the following attributes of the mature pattern of synapsis might arise independently of each other:

- A) The segregation of presynaptic axon terminals in such a way that neighbours belong to neighbouring cell bodies.
- B) The orientation of the set of axon terminals relative to the postsynaptic target.
- C) The positioning of the group of axon terminals in relation to the postsynaptic cells.
- D) The spacing and mutual overlap of axonal arborisations.

(The sequence in which these are listed is arbitrary, not presumptive of any sequence of developmental events.)

Some notes on the terminology applied to these anatomical properties are mandatory to avoid confusion. First, the noun "topography" and the adjective "topographic" will be used to denote the type of organisation achieved by the first of these (A): topography per se connotes no particular handedness nor orientation, no specific position of presynaptic axon terminals, and no characteristic overlap and spacing of axonal arbors. This is in accordance with current usage (Meyer, 1982).

Secondly, the term "orientation" describes the direction of an arbitrary axis, or axes, in the pre-synaptic set of axon terminals relative to an arbitrary axis, or axes, in the post-synaptic set of neurons. It is used without yet knowing whether the relevant mechanisms operate in Cartesian, polar, or some other dimensions. When this is known, current usage will be found to be over-inclusive, and will be modified. Terms in current use which are adopted in discussion of particular models of orienting mechanism, or in description of particular experimental results, include "handedness" and "polarity". In a polar conception of order - e.g. envisaging a two-dimensional set of terminals as a clock face - "handedness" distinguishes that set from its mirror image: clockwise from anti-clockwise. In contrast, in a Cartesian scheme, one might legitimately talk of North - South and East - West axes; reversal (of polarity) of one of these is equivalent to a change of handedness in the two-dimensional whole. It is not certain which format and which terminology will be found to be correct. Current usage of "orientation" is complicated further by uncertainty as to whether this term should incorporate within it the meanings of "handedness" and "polarity": supposing that these describe attributes intrinsic to the pre-synaptic array, only one bit of information is needed to orient that array. For example, the instruction "twelve O'clock uppermost" serves to orient a clock; and a compass pointing North serves to orient a map relative to the ground. But one cannot presume that "handedness" or "polarity" is a property intrinsic to the topographic pre-synaptic array, and if neither is fixed the orienting cue must be more complicated. Use of "orientation", "handedness" and "polarity" is, for the time being, dictated by convenience in respect of each particular context. These overlapping terms are used without apology; rather, with the justification that one may not pre-judge the nature and format of the orienting mechanisms, and must still allow various possibilities.

Lastly, terminology indicating the position adopted by presynaptic axon terminals in relation to postsynaptic cells demands particular attention because of past usage of the word "specific". Sperry (1945, 1948) inferred (incorrectly: see below) from his experiments that each presynaptic cell or group of cells has a "specific" partner in the postsynaptic array of cells with which it invariably establishes contact. This end result was thought to be achieved by a process (termed "neuronal specificity" (e.g. Gaze and Keating, 1972; Hunt and Jacobson, 1974a)) of matching appropriate cells, in which, it was proposed, the operative function might be a cytochemical interaction ("chemoaffinity" or "chemospecificity"). But the topographic arrangement of axon terminals has also been described as a "specific" pattern (i.e. characteristic of the projection (Rager, 1980a)), which might be obtained irrespective of the positions of the axon terminals. Rather than redefine this word, it will be used only where there is no possibility of confusion.

If pre- and post-synaptic cells are in some way paired, the mechanism effecting this selective connectivity automatically juxtaposes the axons from adjacent cells, automatically determines the handedness and orientation of the projection, and also specifies the spacing and overlap of axons' terminal arborisations. Thus all five aspects of the pattern would be achieved by a single organising process. However, this is not the only tenable theory, and although not denying the possibility

that these several parameters might have a common origin it must be emphasized that failure to investigate these five as independent properties would be to prejudge the nature of the organising mechanisms.

The examples of neuronal connections preserving neighbour relations which have been studied most are the retinotectal connections of teleost fishes and anuran and urodele amphibians. The special convenience of studying the retinal projection is the ease with which the projection from selected small areas of retina may be studied, both electrophysiologically (using visual stimuli to evoke electrical activity) and anatomically (both retina and optic nerve being accessible for selective ablative and labelling procedures). Accessibility also favours the study of tectal innervation, for in these fish and amphibia most of the tectum and the caudal part of the optic tract are readily exposed for a variety of experimental procedures.

In these species the retinotectal projection also displays an ability to regenerate and, by so doing, to recover its normal pattern (Gaze, 1959; Maturana et al., 1959) and its function (Matthey, 1925; Sperry, 1943a; Sperry, 1948; Arora and Sperry, 1963; Weiler, 1966). In teleosts (Lanners and Grafstein, 1980) and anurans regeneration involves the regrowth of existing axons (although goldfish are capable of regenerating a retina: Wolberg, 1978); in urodeles a new retina is formed (Griffini and Marcho, 1889; Stone, 1949).

Regeneration of optic fibres provides, in its own right, an example of the formation of an orderly projection, and as such it serves as an experimental paradigm. If it is correct to assume that in their natural environment these species have no requirement for a regenerative capacity in the optic nerve, the possibility that there could have evolved a mechanism specifically for regeneration may be dismissed. Rather, it may be assumed that regeneration invokes a repetition of the same organising principles as those controlling development, or that



events occur in regeneration which by chance have the same effect on the retinotectal projection. However, the argument that the same mechanisms operate in development and in regeneration must remain unproven until both processes are fully understood.

The majority of experiments performed on this system have sought to make a (more or less) controlled change in the conditions under which connections are formed, and to observe consequent changes in the "behaviour" of the regenerating or developing projection. This phenomenological approach has been closely accompanied by the invention and discussion of theoretical models, some of which have been afforded relatively formal mathematical treatment (Prestige and Willshaw, 1975; Willshaw and von der Malsburg, 1976; Hope, Hammond and Gaze, 1976; Fraser, 1980). Elucidation of these mechanisms has not pursued an entirely straightforward course, as noted by recent reviews (Horder and Martin, 1978; Dawney, 1981b). In several important instances interpretations initially placed on experimental results have been abandoned or substantially revised in the light of later experimental evidence: for example, it was concluded that the projection from a partial retina is permanently confined to the appropriate area of tectum (Attardi and Sperry, 1963), but this is not so (Horder, 1971; Yoon, 1972b; Feldman, Keating and Gaze, 1975); and it was believed that the orientation and polarity of the retinotectal projection are entirely labile until the 28th stage of embryonic development in *Xenopus* (Hunt and Jacobson, 1974b), until this too was disproved (Gaze, <sup>Feldman, Cooke & Chung</sup> 1979). While the history of the investigation of retinotectal pattern formation is interesting in its own right, a strictly historical survey of the literature is not ideal for a clear evaluation of current understanding, particularly as models of pattern formation have hitherto failed to distinguish the five parameters of orderliness which, it has been argued above, require separate consideration. It may therefore be preferable to

review the evidence relating to each of these five topics in turn irrespective of the sequence in which the contributions were made, even though this does previous workers the injustice of omitting the reasoning behind each experiment. This policy also necessitates repeated citation of a few experiments relevant to more than one aspect of the retinotectal pattern.

Appreciation of the abnormalities of pattern produced in these experiments will be aided by a brief description of the normal anatomy of the retinotectal projection of the goldfish, which is the species used in the experiments described below. From each retina the axons of approximately  $10^5$  ganglion cells (Murray, 1982) project through the optic nerve, chiasm and tract to the contralateral tectum. (A very small number of ipsilateral efferents has been reported (Springer, 1981), but it has not been shown that these project to the tectum and they may well connect with others of the many nuclei receiving optic innervation (Springer, 1981)). Axons leave the brachia to course over the surface of the tectum in regular array. The ganglion cells form one layer in the retina (Ramon y Cajal, 1893), and their axons terminate in three layers in the tectum (Schmidt, 1979; Meyer, 1980; Stuermer and Easter, 1984b).

The evidence for retinotopy (i.e. topography reproducing the pattern of the retina) is partly electrophysiological: when visually evoked activity is recorded from adjacent sites on the tectum, the corresponding directions in visual space along which a standard stimulus evokes the maximum response, are found to be adjacent to each other too (Jacobson and Gaze, 1964). (By recording the optimum stimulus directions at many tectal loci one may construct a visuotectal map - i.e. a map of the representation of the visual field on the part of the tectum accessible to the recording electrode). There is also evidence from diverse anatomical techniques: autoradiographic labelling of the projections from retinae in which discrete lesions have been

produced (Meyer, 1980); the use of horseradish peroxidase (HRP) to trace the axons from different parts of the retina (Dawney, 1981b); and silver staining to display degeneration resulting from partial retinal lesions (Lazar, 1971). Of these methods, only the electrophysiology maps the projection of the whole retina, rather than that of a selected cohort of ganglion cells. Hitherto there has been no quantitation of the precision of retinotopy. It is therefore not known exactly what is required of developmental processes responsible for the organisation of this projection. Nor is there quantitative evidence that regeneration restores the full precision of normal topography. A new method of quantifying visuotectal maps is proposed in chapter 5.

The retinal connections in each layer of tectum appear to be in register with each other: as a microelectrode is lowered orthogonally through the tectal laminae the direction of the line of optimum stimulation remains the same (Maturana et al, 1959). The projection may therefore be regarded as from a single (curved) lamina of ganglion cells to a single (curved) lamina of tectum without undue simplification. Investigations into the development of orderliness within the retinotectal projection have concentrated on the pattern within the layers and have not sought to understand the control of lamination. The same restriction is also placed on the present work.

Under normal circumstances the orientation of the retinotectal projection is such that dorsal retina connects with ventrolateral tectum, ventral with dorsomedial, nasal with caudal and temporal with rostral (Jacobson and Gaze, 1964); but the precision of this orientation has not been quantified.

The variability of orientation among normal visuotectal maps may carry implications about the mechanism by which it is controlled; and without a method for measuring the effect of experimental interventions upon the orientation of the projection, analysis of this may be

restricted. Chapter 5 describes a means of rectifying this deficiency.

Anurans differ in possessing an additional projection from part of the retina to the ipsilateral tectum (Gaze and Jacobson, 1963), but are in other respects very similar, so it is appropriate to review the literature relating to different species together.

The control of the position of presynaptic optic terminals relative to the tectal cells

When connections are formed between a rotated eye and an unrotated tectum motor responses prove that the frog, newt or fish's sense of visual space is correspondingly rotated: for example, a visual stimulus imaged on embryonically ventral retina placed dorsally is incorrectly interpreted as being in superior visual field (Sperry, 1943b, 1944, 1948). This was interpreted as evidence that each part of the retina reconnects with the same region of tectum as it had innervated before (Sperry, 1948, 1951). But the fact that the animal cannot learn that its response is inappropriate may be because it lacks a higher mental function for analysing the cause of an effect. One cannot assume that each tectal locus corresponds rigidly to a fixed direction of motor response, nor, from that, that the original retinotectal connections are exactly reproduced. A small part of the tectum might be capable of analysing (perhaps with reduced acuity) information carried from a compressed projection from the whole retina (Arora and Grinnell, 1976). The fact that motor responses are inappropriate after eye rotation does mean that the regenerated projection has returned to its original orientation; but not necessarily any more than that.

Abnormal matching of retinal and tectal cells has since been demonstrated by visuotectal and anatomical mapping of projections formed subsequent to removal of part of the tectum (in which case the whole retina forms a compressed projection on to the residual part of the tectum (Gaze and Sharma, 1970; Sharma, 1972a,b; Yoon, 1971)), and following removal of part of the retina (in which case remaining retina forms an expanded projection on an area of tectum larger than it normally occupies (Horder, 1971; Schmidt, Cicerone and Easter, 1978)). Half retinae also form expanded projections when two are surgically united in a compound retina (Gaze, Jacobson and Szekely,

1963). These phenomena are reversible (Yoon, 1972a), and can occur without retinal or tectal tissue being damaged (Yoon, 1972a; Feldman, Keating and Gaze, 1975). The projection can expand or compress in rostro-caudal (Horder, 1971) and medio-lateral (Fraser and Hunt, 1980) directions, and the projection formed by a small retina (hindered from growing by cytotoxic drug treatment) can form a projection expanded radially over the full extent of a normal-sized tectum (Hunt, 1977).

A partial retina may also project to a partial tectum which, embryologically, is wholly inappropriate (Horder, 1971; Yoon, 1972b).

The flexibility of retinotectal correspondence witnessed by these facts may have a physiological role. During development the retina grows radially (goldfish: Johns, 1977; Meyer, 1978; Xenopus: Straznicky and Gaze, 1971) and the tectum grows caudally and laterally (goldfish: Meyer, 1978; Xenopus: Straznicky and Gaze, 1972). Consequently central retina initially projects to the rostral part of the tectum which in the adult receives only temporal retinal innervation. There must therefore be a progressive shift of early connections laterally and caudally (goldfish: Cook, Rankin and Stevens, 1983; Easter and Stuermer, 1984; Xenopus: Gaze, Keating and Chung, 1974; Chung, Keating and Bliss, 1974; Freeman, 1977; Keating, Ostberg & Chung Gaze, 1979; frog: Reh and Constantine-Paton, 1984).

However, this abundant evidence that retinotectal synapses do not involve rigid pairing of individual cells, or even of areas of tissue, contrasts strikingly with diverse other experimental results (see below), from which the converse conclusion has been drawn. This paradox is one of the most interesting features of the subject and compels special consideration.

Tissue excised from caudal tectum and implanted in a rostral position usually (but not invariably) re-acquires an innervation from the same region of retina as originally supplied it (Hope, Hammond and Gaze, 1976), or an equivalent region in the other retina when the translocation

is from one tectum to the other (Jacobson and Levine, 1975). The same result may be obtained when a fragment of tissue is transposed from medial to lateral or vice versa (Yoon, 1977; Martin, 1978a,b). In all these cases it must be assumed that the optic fibres had access to areas of tectum other than those in which they synapsed, and the evidence therefore shows that connections between specific pre- and postsynaptic sites are somehow preferred.

The results of Attardi and Sperry (1963) have also been interpreted in this way: after partial ablation of the retina these authors found that regenerated optic fibres (identified histologically by silver staining) terminated only in those areas of tectum with which they normally correspond. A similar result was obtained by Westerman (1965) and Jacobson and Gaze (1965). These findings do not contradict the fact, discussed above, that the projection from a partial retina can occupy more than its usual proportion of tectum because this expansion can occur subsequent to the initial pattern of terminations (Sharma, 1972a; Yoon, 1976). However, interpretation of the results of Attardi and Sperry, Westerman, and Jacobson and Gaze (opp. cit.) as evidence that regenerating optic fibres actively select their initial termination sites depends on the guarantee that these fibres did have access to, and rejected, other possible tectal loci. Attardi and Sperry reported that the fibres appeared to be confined to their normal parts of the optic tract: whatever the reason for this, it may be that its effect could have been to lead them directly to their eventual termination sites without exposing them to alternatives (Horder and Martin, 1978; Dawney, 1981b). Because of this, these particular results should not be considered adequate evidence of fibres' ability to identify sites on the tectum as they grow across it. (The possibility that the axons possess a faculty for actively selecting pre-ordained growth trajectories is considered in chapters 9 and 10.)

This revised interpretation of the results of Attardi and Sperry does nothing to clarify the discrepancy between the foregoing results, some showing that optic fibres actively prefer particular termination sites and others showing that they can synapse unrestrictedly. The problem is not resolved by proposing that fibres must first synapse at specific tectal positions before migrating under the influence of some other organising process, for in cases of a partial retina connecting with a non-corresponding partial tectum (Horder, 1971; Yoon, 1972) none of the sites of initial contact can be correct. Moreover, it is still disputed whether axons<sup>do</sup><sub>^</sub> form first connections with sp<sup>e</sup>cific tectal loci even when they have the opportunity. Horder and Martin (1977) and Cook (1979) reported that following formation of a compressed projection (of a whole retina on to a partial tectum), if the optic nerve is crushed the ensuing regeneration results initially in a projection in which only that part of the retina which would normally be appropriate makes contact with the tectal fragment. In contrast, in the same experiment performed by Schmidt (1983) the first visuotectal map (obtained after a similar period of regeneration as in Horder and Martin's experiment) encompassed 99% of the visual field: there was no phase of initial reconnection of only normally appropriate areas of retina and tectum. Regeneration of a previously expanded projection also resulted in immediate, rather than delayed, re-expansion (Schmidt, 1978). The discrepancy between these results is still an unresolved bar to their interpretation.

Amidst the perplexity engendered by these contradictory experimental phenomena the most illuminating contribution has been a re-examination of the consequences of tectal fragment translocations (Martin, 1978a,b). Firstly, a piece of rostral tectum placed in a caudal position may become innervated by nasal retinal fibres, which are appropriate to its new site and not its origin. The same is true of lateral tectum placed medially. This concurs with the phenomena of



compression and expansion in demonstrating that any site-selectivity on the part of the optic fibres as they enter the tectum is not exclusive. Secondly, the fibres innervating a fragment of caudal tectum placed rostrally (or lateral placed medially) are in fact not appropriate to exactly the region of tectum from which the graft was taken, but to a position between the donor and host sites. Consequently, axonal selection of termination sites is imprecise. Thirdly, the borders of the area of tectum receiving this misplaced projection do not accurately coincide with those of the grafted tissue; so there are areas of retinotectal connection in which axons synapse adjacent to appropriate presynaptic neighbours but in relation to completely wrong postsynaptic tectal cells. Recognition of specific termination sites is therefore not only facultative and imprecise, it is also less potent an organiser than some other mechanism which generates topography amongst the presynaptic axons. One must conclude that even the experimental paradigm which most strongly supports the idea that axons actively select specific postsynaptic sites, does not justify the proposal that there is a detailed one-to-one matching of retinal and tectal cells (Sperry, 1963).

Numerous examples of partial retinotectal projections which have an abnormal orientation on the tectum are cited on pages 19 and 20. These also refute the idea that the re-establishment of the retinotectal projection is governed by a fixed correspondence between retinal and tectal loci. Furthermore, they compel rejection of modified chemo-affinity models explaining the detailed arrangement of connections (a) in which a gradient of positional values in retina and tectum mediates the matching of one set against the other without specific pairing of the component parts of each (Gaze, Jacobson and Szekely, 1963; Gaze, 1970); and (b) in which topography is determined by positional markers which can be moved and induced by the optic axons themselves (Meyer and Sperry, 1976; Schmidt, 1978).

Undeniably, the position adopted by an array of optic axon terminals can be influenced by interaction between it and the tectum (e.g. Hope, Hammond and Gaze, 1976); and it may be that this occurs through the medium of cell surface molecules (Sperry, 1963). But such interactions are weak and do not dictate the positions on the tectum of individual fibres.

The discovery that tectal grafts translocated to foreign sites can become innervated by fibres retinotopically appropriate to the surrounding axons (e.g. Martin, 1978a,b) supports the suggestion that fibre interactions might contribute to the pattern of connections. This possibility complicates the interpretation of such experiments; and may be avoided either by studying the ability of a small cohort of axons to locate their appropriate destination in a tectum devoid of other innervation; or by studying the influence of tectal cells on the growth of axons in vitro. Fibres re-directed from their normal termination sites in postero-lateral tectum (on the contra-lateral side) to the antero-medial quadrant of the ipsi-lateral tectum (previously denervated for up to 18 months), were subsequently located by autoradiographic and electrophysiological means and were found to have grown more into lateral tectum than medial; but showed little preference for posterior regions (Meyer, 1984). When fibres of temporal retinal origin growing from a compound eye were diverted into unoccupied ipsi-lateral tectum, their projection was limited to the rostral area: another indication of at least crude selectivity (Straznicky and Gaze, 1982). But fibres of nasal origin showed no preference for caudal tectum under similar conditions.

Using an in vitro assay which presents growing chick optic neurites with a choice between substrates composed of cells from different parts of the tectum, it has been shown that axons from temporal retina grow over cells from rostral tectum in preference to those from caudal (Bonhoeffer and Huff, 1982). This corresponds to the normal retinotectal

relationship. The converse preference of nasal retinal axons for caudal tectum was not observed; but whereas temporal fibres prefer retinal cells when offered a choice of retinal or tectal, the nasal fibres prefer tectal (Bonhoeffer and Huff, 1982). Tectal cell membranes also adhere more to neurites from anterior retina than to those from posterior retina (Halfter, Claviez and Schwarz, 1981). Further study of neurite growth in vitro might show the resolution of axonal discrimination between tectal loci. It might also be possible to interfere with this interaction by specific biochemical techniques (perhaps surface-binding immunoglobulins) and thus identify the mechanism of this process.

An earlier assay compared the adhesiveness of mixed retinal cells for tectal cells of anatomically appropriate and inappropriate origins (Barbera, Marchase and Roth, 1973; Barbera, 1975; Pierce, Marchase and Roth, 1978). The cell surface interactions involved in this might bear no relation to synaptogenesis in vivo between specific parts of specific types of cells. But it is noteworthy that cells from dorsal neural retina adhere preferentially to cells of the ventral half of the tectum and vice versa (Barbera, Marchase and Roth, 1973; McClay, Gooding and Franson, 1977; Gottlieb and Glaser, 1980).

Results of both types of experiment (re-direction of fibres into vacant tectum, and retino-tectal affinities in vitro) show that fibres do express, by their growth, a preference for specific tectal loci; but at present this appears to be of only low resolution. Discovery of retinotopic asymmetry in the effects of treating cultured retinal or tectal cells with galactosaminidase and galactosidase (Marchase, 1977) may be the first step in identifying the biochemical basis of these phenomena.

It remains to consider the role of a mechanism matching a part of the optic projection, however crudely, with a part of the tectum. It is clear from the foregoing discussion that this is not the sole organising

process and is not responsible for dictating the neighbour relationships of optic terminals: retinotopy is a pattern intrinsic to the fibre array, independent of tectal location. The fact that optic terminals shift caudally after their initial synapsis makes it seem unlikely that there could be a role for a mechanism leading axons to particular parts of the tectum. Teleological reasoning is not without hazard; but it is interesting to consider whether tecto-axonal interactions have an entirely different function, which is to orient the optic projection on the tectum; and that all the examples of imprecise "selectivity" of postsynaptic targets occur simply because of the particular nature of the orienting mechanism.

## The control of orientation in the retinotectal projection

After severance of the optic nerve and rotation of the eye in situ in adult newts, frogs or teleosts, the retina reforms connections with the tectum according to its original orientation and not its new (Sperry, 1943a,b; 1948). Similarly, after exchanging the positions of left and right eyes without rotation each connects with its new contralateral tectum in a manner appropriate to its original position: the effect is to reverse only the horizontal axis of visuomotor responses (Sperry, 1945). Combining left/right exchange with 180 degree rotation predictably results in a projection with <sup>its</sup> original orientation, so that the vertical axis of visuomotor responses is reversed. The concept of Cartesian axes in horizontal and vertical planes has a descriptive convenience in these experiments, but the optic projection need not be Cartesian in its construction. The same results could equally be expressed as reversals of orientation and /or handedness within a circumferential dimension. Thus, the handedness and orientation of the array of optic terminals are not fixed relative to the eye by a rigid arrangement of fibres within the optic nerve and tract, but are actively matched with the half of the brain to which the retina projects. This property has been shown to be established before the appearance of post-mitotic ganglion cells (Sharma and Hollyfield, 1978). (The unsatisfactory inconstancy of terminology applied to orientation has been excused on page 2a.)

Many experiments have been performed in which developing eyes were transferred to ectopic sites (e.g. the flank of the tadpole: Hunt and Jacobson, 1972; Hunt and Berman, 1975; Hunt and Piatt, 1978) or to culture environment (Hunt and Jacobson, 1972) for part of their development, and re-implanted in an orbit later on. In some of these, and (Nieuwkoop and Faber, 1956) in some simple eye exchanges up to stage 32, the retinotectal projection formed has the orientation and handedness appropriate to the <sup>intermediate or</sup> final host site (Jacobson, 1967, 1968). But this does not necessarily indicate respecification of these parameters because the eye may contain not donor

retinal tissue but neurons which have entered the eye subsequent to its implantation in the host (Holt, 1980). This provides a simple explanation for the finding that when wild-type eye anlagen are grafted in rotated orientation into albino host orbits some of the corresponding tecta receive not only a rotated retinal input (appropriate to the graft) but also a normal one, which could come from host tissue (Gaze, Feldman, Cooke and Chung, 1979; Munro and Beazley, 1982).

When part of the tectum is removed, rotated and grafted back the part of the overall projection received by that fragment of tectum has a correspondingly rotated orientation (Sharma and Gaze, 1971; Yoon, 1973, 1975; Levine and Jacobson, 1974). This occurs even when the part of the retina connecting with this fragment is not that which would normally project there (Yoon, 1976; Martin, 1978b), so the effect is not due to detailed recognition by axons of specific tectal sites. These results indicate that the interaction determining orientation is between the optic axons and the tectum itself, for these operations do not disturb the diencephalon or other structures. However, phenomena discovered by Chung and Cooke (1978) contradict this: if dorsal thalamus in the embryonic *Xenopus* is divided into two and one part placed in front of the tectal precursor and the other behind, superimposed visuotectal maps with opposing polarities are subsequently found in the adult. Further experiments involving diencephalic tissue translocation to the middle of, or behind the tectum also resulted in optic projections with reversed rostral-caudal polarity (Chung and Cooke, 1978) showing that the position of the diencephalon can over-ride the tectum as the major determinant of at least the anterior-posterior polarity of the visuotectal map. The fact that diencephalon fails to dictate the orientation of tectal innervation during regeneration in the adult might be because the diencephalon changes with maturation, or may be because regenerating optic fibres do not grow to the tectum in intimate contact with diencephalic cells

(Xenopus: Gaze and Grant, 1978; goldfish: Dawney, 1981a) in the way that the first fibres do in development (Gaze and Grant, 1978; Dawney, 1979b). The most curious aspect of these discoveries is the duplication of function: there is no apparent need for the tectum to be able to control the orientation of the developing projection if the diencephalon is similarly and more potently empowered; nor for there to have evolved a separate mechanism to control orientation in regeneration, in a species presumed not to require this faculty in its natural state.

Attempts have been made to learn something of the mechanism controlling orientation by identifying conditions in which it does not operate. Numerous instances have been reported in which parts of retinae project with an orientation or handedness different to that expected from the embryonic position of the tissue. Many of these follow surgical combination of parts of different retinae (Hunt and Jacobson, 1973; Hunt and Frank, 1975; Ide and Hunt, 1978; Conway, Feiock and Hunt, 1980; Cooke and Gaze, 1983; Willshaw, Fawcett and Gaze, 1983) which duplicates some embryonic positional values within the compound eye. Other examples occur in compound tecta constructed from two rostral or two caudal halves (Sharma, 1975) or two lateral halves (Rho and Hunt, 1980). In such cases mechanisms governing the orientation of individual axonal arrays on the tectum might be over-ruled by interactions between fibres - discussed below. Abnormally oriented partial projections may also result from simple bisection of an embryonic retina: the two halves may form duplicate mirror image projections (Hunt and Jacobson, 1974b). Although these cases involved no intentional alteration of tissue positions, it has been pointed out that interference with developing tissues might invoke ill-understood regenerative and regulative processes (MacDonald, 1975, 1978; Ide, Kosofsky and Hunt, 1979; Ling, Ide and Hunt, 1979; Cooke and Gaze, 1983) with the potential to alter positional values without the tissue actually changing place.

Reversal of the rostro-caudal polarity of part of a retinotectal projection has been observed to follow excision of the caudal half tectum (without the optic axons being otherwise disturbed) (Horder and Martin, 1977). It is not clear why this should occasionally happen as an alternative to compression of the projection into a coherent pattern on the residual tectum. Surgical rotation of only the peripheral part of the eye may also be followed by the formation of an un-rotated projection from peripheral retina to the tectum (Burgen and Grafstein, 1962). Reversal of part of a visuotectal map has also been reported intermittently when rotation of a fragment of tectum has not been followed by corresponding rotation of the projection formed on it (Martin, 1978; Bunt, Horder and Martin, 1978), and when an anomalous projection to the ipsi-lateral tectum is induced (Cunningham and Speas, 1975: reversal of the medio-lateral axis in the rat; Meyer, 1979a: reversal of the antero-posterior axis in the goldfish). Reversal of part of a retinotectal projection has been achieved reproducibly only by simultaneously ablating temporal retina and caudal tectum, leaving intact a narrow mediolateral band of optic connections formed by the vertical meridian of the retina: the remaining nasal retina, dispossessed of its caudal tectal target, forms on the vacated rostral tectum a new projection as if its old pattern were reflected about the line of intact connections (Martin, 1978).

Experiments on retino-tectal interactions in vitro (described above) indicate preferential cellular adhesiveness. To provide only enough information to dictate the orientation and handedness of the projection it is not necessary for there to be a gradient of adhesiveness extending throughout the tectum: one unipolar affinity could specify orientation (cf. "12 O'clock uppermost"); two (not diametrically opposite) could fix the handedness of the projection too (cf. MacDonald, 1977). The experimental data does not indicate the existence of linear



gradients of affinity, but this interpretation has been advanced (Gottlieb and Glazer, 1980; Halfter, Claviez and Schwarz, 1981; Bonhoeffer and Huff, 1982).

Clearly information about the control of orientation is still fragmentary. One problem is that there is no exact definition of what constitutes normal orientation, nor quantitative confirmation that the described errors are 180 degree reversals of polarity. Adoption of the mathematical analysis proposed in chapter five might help discover the conditions under which orientation is optimal, impaired and reversed.

### Control of axon terminal spacing and overlap

Visuotectal maps of *Xenopus* tadpoles show that the entire retinal projection occupies only the rostral half to two thirds of the tectum, and that there is a relatively expanded representation of temporal visual field; but in the adult the entire dorsal surface of the tectum is occupied, and the projection is (to a first approximation) uniform (Gaze, Keating and Chung, 1974), as in the adult goldfish (Jacobson and Gaze, 1964). The concept of competition between fibres for tectal space has been invoked to explain this, and to account for the tendency of a retinal projection to take up all the space available on the tectum (Martin, 1978a; Bunt, Horder and Martin, 1979). An alternative concept is that of mutual repulsions between fibres (Fraser, 1980); but there is no information about how such processes might be effected, in terms of cell behaviour.

In compressed retinotectal projections examined three months to four years after ablation of caudal tectum the number of synaptic contacts per column through the principle termination layer (Stratum fibrosum et griseum superficiale) is the same as in the normal projection (Murray, Sharma and Edwards, 1982). These authors calculate that if the proportion of terminals which are optic does not change, the number of terminals per axon falls by 40%. Competition between axons for limited synaptic sites may explain this too.

Quantitative confirmation of the uniformity of projections formed under normal and abnormal conditions would provide an additional indication of the existence of this type of regulatory mechanism: results presented in chapters 5 and 6 are important in this respect.

Some attention has been given to the interaction of different populations of axons, for example, from left and right eyes, simultaneously innervating the same tectum. Sometimes their projections are simply superimposed on each other (Hunt and Jacobson, 1974a); but a

curious phenomenon of "patching" or "banding" may also be observed, in which the two groups segregate from each other into abutting islands or strips of terminals (Cronly-Dillon and Glaizner, 1974; Levine and Jacobson, 1975; Meyer, 1979b), provided that at least one projection is electrically active (Meyer, 1982a). The same has been reported in the superimposed projections formed by the two halves of a compound retina (Fawcett and Willshaw, 1982), which suggests that the phenomenon is not an expression of immiscibility of axons from left and right sides (such as might be required to prevent the intermingling of fibres at the chiasm); so it may be a manifestation of a form of axonal interaction which contributes to the partition of tectal space between the components of a normal projection.

It has been noted that a double-nasal compound retina innervates more of a "virgin" (previously unoccupied) tectum than will a double-temporal eye (Gaze and Fawcett, 1983). Similarly, sectors of nasal retinal origin grafted into a temporal position in a host retina form projections to larger areas of tectum than similar sectors taken from temporal retina and placed in a nasal position (Willshaw, Fawcett and Gaze, 1983). Thus, there may be broadly retinotopic differences in whatever aspect of axonal behaviour regulates the spacing of fibres.

## Generation of topography in the presynaptic array of axons

The foregoing discussion has shown that elements in the retinotectal projection may relatively easily be made to connect with areas of tectum other than those with which they normally correspond (page 9). Under some circumstances the projection may also adopt an abnormal orientation (pp. 19-20): although few, these cases are most important, for they demonstrate that even when site and orientation are incorrect, topography is maintained.

This compels the rejection of any theoretical model in which topography is dependent on active matching of individual retinal and tectal loci - for example, by means of cytochemical labels on pre- and postsynaptic cells conveying positional information (Sperry, 1963). Such labels might exist, though perhaps not uniquely marking individual cells; they might be changeable (Schmidt, 1978; Willshaw and von der Malsburg, 1979); and they might be altered by optic innervation (Schmidt, 1978; 1983). But their role can only be to control the orientation and general position of the array of optic terminals, not to enforce a detailed pattern of connections. Topography, the detailed imitation by the pattern of retinotectal terminals of the neighbour relationships between ganglion cells, must be a property intrinsic to the projection and not imposed on it by the tectum.

Models of axonal behaviour attempting to explain topography may be classified into two groups:

- (a) "Active" mechanisms, in which axons discriminate between retinotopically correct and incorrect positions in the presynaptic array;
- (b) "Passive" mechanisms, in which fibres have no option but to terminate topographically, because of spatial or temporal constraints.

The "active" class comprises two possibilities. One is that axons carry information indicating which part of the retina they come from, so that by mutual exchange of this information they are able to identify appropriate neighbours. This information might be coded in the form of chemical labels (Sperry, opp.cit.) or as a pattern of electrical activity

(Chung, Gaze and Stirling, 1973). The second possibility is that axons juxtaposed initially by chance interact, mutually strengthening otherwise transient connections with the tectum if they happen to originate in neighbouring areas of the retina. The information required for this concerns the proximity of ganglion cells to each other, not their absolute positions on the retina. Here, too, the information might be conveyed electrically or chemically (Willshaw and von der Malsburg, 1976). The process of sorting in both these models could occur during the axons' growth through the optic pathway and in the tectum itself.

The "passive" class of mechanism also has two important subdivisions, for an axon could be restricted to a particular site by limitations of space or limitations of time. In the former category, if optic fibres retain throughout their growth the neighbour relations of their cell bodies their terminations in the tectum will inevitably be topographic (Horder and Martin, 1978). Alternatively, if there is a retinotopic temporal sequence in their arrival at the tectum, one need postulate only that consecutive axons should synapse adjacent to each other for this sequence to constrain them automatically to a topographic pattern of connections. Although this was conceived by 1960 (Gaze, 1960) it has not received much attention. This model could operate in various ways. One suggestion is that if axons leave the fascicles coursing over the tectum in a sequence reflecting the relative positions of their origins in the temporo-nasal axis of the retina (i.e. "temporal first, nasal last"), this could contribute the rostro-caudal topography of their terminations (Stuermer and Easter, 1984). At present this does not appear to be a plausible explanation of order in the regenerated map, because axons can re-grow along abnormal, contorted routes over the tectum (Cook, Pilgrim and Horder, 1983). Another possibility is that sequential arrival of axons from retinal annuli of increasing radius could produce the correct radial dimension in the map of terminations.

Because the last of these various models relies on time it can generate only one dimension of topography; but there is no prima facie reason why a single mechanism must be responsible for both dimensions of the map (contrary to earlier assumptions: e.g. Prestige and Willshaw, 1975). Equally, there is no reason why more than one mechanism should not combine together within each dimension to produce a degree of precision of which neither alone is capable.

The simplest of these models is that topography among optic axon terminals arises passively as a consequence of preservation of topography throughout the optic pathway (Horder and Martin, 1978). It is a general property of neurites that they grow along other axons (Harrison, 1910; Wigglesworth, 1959; Halfter and Deiss, 1984). This probably does not require exchange of "positional information" since they similarly grow along inanimate lines in their substrate (Weiss, 1941). Weiss and Horder and Martin (1978) have termed this property "contact guidance". It may be that this phenomenon also occurs in growing optic axons (Bodick and Levinthal, 1980). Within the retina these fibres converge on the optic papilla, growing over and along each other (Bunt, 1982). Orderly growth in the extraocular path of these axons is suggested by their generally parallel arrangement in the optic nerve and tract (Bunt, 1982; Fawcett and Gaze, 1982), but this appearance might be partly attributable to elongation of the optic pathway after the formation of tectal connections.

The architecture of the goldfish optic tract is retinotopic (Dawney, 1979b): fibres from central retina lie medially in the deepest part of the tract, those from peripheral retina lie superficially; and the dorso-ventral sequence is of fibres originating in nasal, ventral, temporal, dorsal, and again nasal quadrants of the retina. Although this appears, at first sight, to invite the conclusion that optic axons have preserved retinotopic neighbourliness throughout their course, the

topography in the optic tract has a layout which is dissimilar to that in the optic nerve head (ventral, nasal, dorsal, temporal, ventral). Between these points there must have occurred a rearrangement in which certain neighbour relations were temporarily lost. A similar conclusion applies to the frog (Scalia and Arrango, 1983). Comparable rearrangement occurs in the optic pathway of the cichlid "Dempsey" fish: for this a schematic model has been conceived (Scholes, 1979), in which topography is preserved within but not between the projections of different sectors of the retina. Experimental evidence about the extent to which orderliness is maintained within each sector in goldfish and *Xenopus* is considered in chapters 9 and 10.

Both the optic nerve and optic tract display radial retinotopy, so contact guidance may be a plausible explanation of this dimension of order amongst the optic terminals. However, contact guidance is not essential for the radial topography of a regenerated projection, for disorganization of this dimension of fibre arrangement (by cutting the optic nerve) does not similarly disorganize the subsequent retinotectal map (Dawnay, 1981b).

The possibility that contact guidance accelerates the acquisition of retinotopy among the connections is not excluded by this discovery, but contact guidance alone is not an adequate explanation for orderliness. Fish mapped six months after an attempt to cross the brachia of the optic tract were judged to have less regular topography in their retinotectal connections than normal fish (Cook, Pilgrim and Horder 1983). The appearance of "some signs of gross re-arrangement [dis-arrangement] of groups of terminals" persisted for at least 5 months more, which suggests that secondary refinement of topography has limited power; and that even if contact guidance and the normal arrangement of axons in the optic tract are not necessary for topography of fibre terminations, an abnormal bias of tract architecture can be detrimental

to the map.

Experiments in which a cohort of axons from central retina is traced anterogradely by labelling with horseradish peroxidase have also excluded the possibility of active restoration of radial orderliness by reseggregation of axons within the optic tract during regeneration. These fibres can be seen to be distributed throughout the cross-section of the optic tract up to the axons' entry into the tectum (Dawney, 1981a). If there is a mechanism by which fibres actively select their radially-appropriate neighbours, it must occur only on the tectum.

The possibility that fibres reseggregate in the optic tract according to the circumferential dimension of topography is considered in chapters 9 and 10.

Active axonal selection, on the tectum itself, of correct pre-synaptic neighbours might be reflected in a progressive refinement of orderliness. Multi-unit receptive fields (mapped by recording visually evoked responses simultaneously from several axon terminals in the tectum) are abnormally large soon after regeneration and subsequently diminish to their normal size (Schmidt and Edwards, 1983). Anatomical evidence of progressive refinement of the regenerated projection has been obtained by labelling the axons from all but a small area of retina with tritiated proline, and examining the tectum by autoradiography (Meyer, 1980). In the normal projection the corresponding area of tectum is devoid of silver grains. After 41 days of regeneration a large retinal lesion produces an appropriately placed but incomplete diminution in tectal labelling. After 69 to 118 days of regeneration the area of unlabelled tectum had indefinite edges; 149 days resulted in a normal pattern of <sup>restricted</sup> labelling. Early in regeneration a lesion of the nasal half of the retina reduced the labelling of caudal tectum: remaining silver grains were arranged in strings - probably indicating that the label was in axons not their terminals (Meyer, 1980). Unless the intra-cellular



distribution of the label is examined the method is unsuitable for assessing the retinotopy of connections. Murray and Edwards (1982) reported that the number of unmyelinated axons in the stratum fibrosum et griseum superficiale (S.F.G.S.) is twenty times normal at the time when the topography is poor (by Meyer's criteria), but the density of synapses is low. This increase is caused by axonal sprouting, not by formation of new ganglion cells (Murray and Forman, 1971). Later the number of axons diminishes, concurrently with the apparent refinement. It has not been shown that the elimination of axons is retinotopically selective; but there could be a mechanism selecting those axons which may form synapses, rather than eradicating connections which have formed at erroneous sites. The time course of these changes is compatible with the theory that organization is achieved by interactions between fibres within the S.F.G.S.

Development of the retinotectal projection in the chick is accompanied by death of some ganglion cells. It has been proposed that this matches the size of the retinal input to the size of the tectum (Rager and Rager, 1978), but it could eliminate errors of topography. Errors induced experimentally are removed (McLoon, 1982) by the degeneration of axons (Fujisawa, Thanos and Schwarz, 1984), which occurs at the same time as in normal chicks (Rager and Rager, 1978). One method of looking for similar changes in the regenerating optic projection of the goldfish would be to study changes in the pattern of arborization of individual axons, injecting intracellular horseradish peroxidase at different stages in the maturation of the projection. This <sup>technique</sup> has already been achieved in normal (non-regenerated) axons (Stirling and Merrill, 1984) in frogs.

Other experiments have been devised to test predictions made from specific varieties of "active selection" model. The possibilities that patterns of electrical activity could convey positional information

(Chung, Gaze and Stirling, 1973; Chung, 1974; Arnett, 1978; Arnett and Spraker, 1981), or that this activity could be instrumental in strengthening the synapses formed by adjacent axons when these belong to neighbouring ganglion cells (Willshaw and von der Malsburg, 1976), may be jointly tested by elimination of impulse conduction in the optic fibres. Harris (1980) transplanted an eye rudiment from a Mexican Axolotl (which is sensitive to tetrodotoxin (TTX)) to a Californian newt (which produces it). This toxin is a selective inhibitor of sodium channels in neural membranes and abolishes sodium-dependent nervous activity. Anatomical mapping of the retinotectal projection subsequently formed showed no less precision than the normal.

In a variation on this theme, Harris (1984) grafted eye primordia to ectopic sites in Axolotl embryos (the hosts being genetically eyeless or enucleated), and united these parabiotically with Californian newts before optic axons emerged from the eyes. TTX produced by the newt paralysed the axolotl and abolished electrical activity in the developing optic fibres. HRP injected into the retina one week later (early larval stage) showed a projection from dorsal retina to ventro-lateral tectum and from ventral retina to dorso-medial tectum: evidence that neither electrical activity nor a normal optic pathway are essential for rudimentary topography.

A similar experimental paradigm has been used by Meyer (1983): tetrodotoxin was injected into goldfish for all or part of the duration of regeneration and was shown to abolish all detectable activity in the optic fibres. Topography in the resulting projection was assessed autoradiographically: a partial retinal lesion of variable size was produced, and tritiated proline injected into the eye. Absence of anterogradely transported isotope from localized areas of tectum indicated hemiretinal topography; but smaller retinal lesions produced no gaps in the labelling of the tectum: the accuracy of topography was

therefore less than normal. The conclusion that "activity sharpens the map" is greatly strengthened by the observation that inactivation of axons with TTX diminishes their ability to sprout into electrically silent areas of tectum (Schmidt, 1982): this excludes an alternative explanation of the impairment of topography, which is that initially topography may have been as precise as ever, only deteriorating secondarily as axons sprout into electrically silent (and perhaps apparently vacant) tectum.

TTX has also been found to prevent the normal diminution in multi-unit receptive field size (from  $30^\circ$  to  $10^\circ$ ) which occurs with the maturation of a regenerated projection (Schmidt and Edwards, 1983).

Other experiments have attempted to create abnormal patterns of impulse activity in optic axons by subjecting fish and amphibia to continuous stroboscopic lighting during regeneration of the optic nerve. One report indicates no loss of topography (Chung, Gaze and Stirling, 1973); another shows that irregular flash lighting retards or prevents the refinement of the visuotectal map normally observed (Cook and Rankin, 1984a), and concludes, with Meyer, that activity somehow contributes to the refinement of crude topography (contrary to the result from Harris's anatomical study (1980)). These reports are agreed on the fact that at least one other mechanism must be operating in conjunction with this in the establishment in the projection.

A very different experimental system has recently contributed to evaluation of theories of "active selection" by fibres of retinotopically correct neighbours in the pre-synaptic array. This is the mapping of tectal connections formed by "compound" eyes. Half retinae with similar origins combined in one eye form projections superimposed on each other, with the corresponding parts of each half in register (Straznicky and Gaze, 1980). Narrower sectors grafted into abnormal sites (creating "pie slice" compound eyes) generally form projections superimposed on that

part of the host projection corresponding to the origin of the graft (Willshaw, Fawcett and Gaze, 1983). Such results are evidence for the existence of positional markers in the retina, which are responsible for the topography of the projection.

Not all the results obtained from this type of experiment readily conform to this interpretation. Compound eyes in which the grafted tissue has an origin different from that of the host half may form normal projections (Gaze and Straznicky, 1980); so do some "pie slice" compound eyes (Cooke and Gaze, 1983; Willshaw, Fawcett and Gaze, 1983). Genetic marking of the retinal pigment epithelium underlying such grafts (by the use of albino/wild type combinations) indicate that the grafts have probably survived in such cases (Cooke and Gaze, 1983; Willshaw, Fawcett and Gaze, 1983). Even better is the use of the heterozygous Oxford Nucleolar marker which labels the neural tissue itself, and supports the same conclusion (Conway, Feiock and Hunt, 1980). These results suggest that there is alteration of retinal labels (see page 19); and the possibility of this makes it difficult to draw inferences from these particular maps about the mechanism responsible for axon terminal topography.

In the context of these discoveries, it is important to consider exactly what the role of retinal positional markers can be. During development the retina grows by the addition of new cells at its perimeter (Johns, 1977; Meyer, 1978), and one may assume that there is consequently a central-to-peripheral sequence in the arrival of their axons at the tectum. As each successive annulus of ganglion cells forms tectal connections, the choice of presynaptic neighbours could be strictly limited to the circumferential dimension: the radial topography of axonal terminations could arise automatically without reference to positional information, if as each axon arrives it can synapse only at the edge of the set of pre-formed connections. It is recognised that the

radial retinotopy of the normal optic tract arises from sequential growth of axons (Dawney, 1979b; Easter, Rusoff and Kish, 1981): the proposition that the same dimension of the axon terminals' topography might arise in the same way is attractively simple and economical. This is the neglected "passive" model: either confirmation or disproof of its contribution to the retinotectal map would be most valuable.

It is difficult to alter the sequence of arrival of optic axons at the tectum during development in order to examine the significance of this sequence for radial topography. Hunt (1975a) altered the time of arrival of developing axons in *Xenopus* by administering fluorodeoxyuridine. This produced a three week cessation in the formation of new ganglion cells at the perimeter of the retina; but when growth resumed it did so with no disturbance of the radial sequence in which cells are produced, so there was presumably no change in the sequence of arrival of their axons at the tectum. (Topography was unaffected.)

Regeneration provides another opportunity to assess the contribution of this passive mechanism of generating topography within the array of axons. In the newt, regenerated central retina is older than regenerated peripheral retina (Gaze and Watson, 1968), and there is a central to peripheral sequence in the regeneration of the projection to the tectum (Cronly-Dillon, 1968). But in goldfish and anurans it has been assumed hitherto that fibres re-invade the tectum either synchronously (e.g. Prestige and Willshaw, 1975), or at least with no retinotopically defined sequence. No experiment has tested the validity of this assumption.

It is likely that all the axons start to regenerate at the same distance from the tectum; but this is no guarantee that they start to grow at the same time, nor that they grow at the same rate. The nature of the signal to the ganglion cell nucleus initiating the response to axotomy has not been identified. One model proposes a retrograde signal

passing up the axon from the site of the lesion (Cragg, 1970), from which one might predict that the time lapse before regeneration starts will depend directly on the length of axon remaining. This is observed in the response to axotomy shown by the hypoglossal nerve of the rat: swelling, and increase of ribonucleic acid production occur sooner if the lesion is close to the cell body (Watson, 1968). Another factor may be that the growth of axons takes place at, or close to, the growing tip; and one can again speculate that for a longer axon stump it would take longer for the first products of regenerative metabolism to reach the growing end.

It is essential to discover the sequence in which reinnervation of the tectum occurs, and this is the purpose of the experiment described in chapters 3 and 4. If the sequence of arrival of axons in regeneration is shown to be centrifugal, this process must be considered a recapitulation of development in a hitherto unsuspected way. If there is a retinotopic sequence, does alteration of this sequence have a reproducible effect on the radial topography of the resulting map? If so, sequential arrival of axons at the tectum must be a phenomenon necessary to the formation of radial topography. Following that must be the inquiry whether this by itself is sufficient to account for the precision of topography, or whether it must act in synergy with other mechanisms.

There could, moreover, be significant repercussions for other models of the generation of orderliness: any contribution to radial topography encourages interpretation of the entire map in polar dimensions. (This has already occurred in respect of the organisation of the optic pathway: Dawney, 1979b; Easter, Rusoff and Kish, 1981; Bunt, 1982.) General conclusions about the importance of locus-specific labels (e.g. Willshaw, Fawcett and Gaze, 1983), and about the necessity for neuronal activity in the evolution of refined topography (Schmidt and Edwards, 1983) might need to be reconsidered in radial and circumferential dimensions separately. Possibly debate about control of

the orientation of the map also ought to change from concern with antero-posterior and medio-lateral preferences to consideration of angular orientation.

Should it be found that sequential arrival of regenerating axons plays no part in the creation of topography, this result would also have considerable importance: a final rejection of "passive" models could be made, and attention re-directed to the "active" models discussed above.

## CHAPTER TWO

### MATERIALS AND METHODS

1. FISH
2. SURGICAL PROCEDURES
  - 2.1 Anaesthesia
  - 2.2 Equipment
  - 2.3 Transection of the optic nerve
  - 2.4 Transection of the optic tract; application of HRP
  - 2.5 Punctate retinal lesions; application of HRP
  - 2.6 Post-operative survival
  - 2.7 Perfusion and fixation of fish
  - 2.8 Preparation of histological sections
3. HORSE RADISH PEROXIDASE HISTOCHEMISTRY
4. MICROSCOPY AND DRAWING OF HRP - LABELLED TISSUE
5. COUNTING CELLS
6. VISUOTECTAL MAPPING



## 1. FISH

Goldfish (*Carassius auratus*) 4 - 8 cm from mouth to base of tail were supplied by local dealers and kept in glass aquaria or opaque lidless plastic tanks, at room temperature (fluctuating seasonally between about 5 C and 20 C and in a normal light - dark cycle. They were fed thrice weekly on Phillips' flaked fish food and remained constant in length.

On delivery the fish were transferred to saline (5 g/l NaCl in tap water for one day, then 9 g/l NaCl for four days, 5 g/l NaCl again for one or two days, and finally returned to tap water) to remove leeches and to treat a fungal infection of their scales. 0.1 g/l Chloramphenicol sodium succinate ("Chloromycetin", Parke - Davis) was routinely added for one or two days for further treatment and prophylaxis of fungal infections. The water was aerated intermittently and changed weekly.

The relevant anatomy is illustrated diagrammatically in figure 1.

## 2. SURGICAL PROCEDURES

### 2.1 Anaesthesia

All operations were performed under anaesthesia produced and maintained with ethyl-p-aminobenzoate ("Benzocaine", Sigma chemical company). The solubility of this in water is pH - dependent; a saturated solution in doubly-distilled water (pH = 6.4) was prepared fresh for each operation. Anaesthesia was induced by transferring fish to a half - saturated solution until gill and eye movements ceased and the righting reflex was lost. For long operations the fish were wrapped in wet tissue paper. For surgery each was positioned on a metal hammock and held gently with "plasticene" modelling clay. The gills were superfused at 40 to 60 ml/min with aerated benzocaine solution at one thirtieth of the saturated concentration: this was found empirically to maintain anaesthesia. After surgery superfusion continued with tapwater until the restoration of

rhythmic gill movements, eye movements and righting reflex. The fish were then returned immediately to tap water.

## 2.2 EQUIPMENT

Surgery was performed under a Zeiss operating microscope using unmounted scalpel blades, fine forceps (Dumont No. 5, TAAB), iridectomy scissors (Weiss), and hypodermic and mounted needles.

## 2.3 TRANSECTION OF THE OPTIC NERVE

The right eyeball was lifted out of, or rotated within, the orbit using forceps which were then used to tear the conjunctiva, exposing the optic nerve from the dorsal or ventral aspect as required. Fluid in the orbit was removed by absorption into small twists of tissue paper. The nerve was then cut either mid-orbitally or juxta-sclerally.

The mid-orbital lesion (at "A" in figure 1) was a complete orthogonal transection performed from the ventral side using scissors, avoiding damage to the blood vessels alongside the nerve and leaving the tenaculum intact. A hooked needle was passed through the lesion to verify that the nerve and perineural sheath were completely severed, and was used to tease the fibres proximal to the lesion, further disrupting their normal arrangement. This lesion was made prior to regeneration in all except the normal fish in each experiment.

The other nerve lesion used was a selective cut through only the dorsal part of the nerve adjacent to the sclera (at "C" in figure 1): it therefore transects only fibres originating in central retina (Dawney, 1979b). This lesion was made either with scissors (tenaculum also cut) or with a hooked needle (tenaculum left intact).

Immediately following either of these lesions the eyeball was returned to its original position.

#### 2.4 TRANSECTION OF THE OPTIC TRACT; APPLICATION OF H.R.P.

A scalpel blade was used to incise all but the anterior side of an octagonal suture in the fish's cranium, and the bony plate thus delineated was reflected forwards using the skin as a hinge. Because the incisions were bevelled the plate could be clipped back into place after surgery and held by the surrounding bone without cement. During the reflection of the bone plate the meninges adherent to it were carefully separated from the subjacent midline dorsal sinus. Blood vessels on the surface of the brain were undamaged and bleeding from other vessels was transient and never fatal.

Fluid surrounding the brain was absorbed with tissue paper twists, one of which was also used to gently displace the left lobe of the telencephalon anteriorly. This exposed the left optic tract and its brachia lying on the lateral surface of the diencephalon in the cleft between the telencephalon and the tectal lobe. The tract was cut orthogonally close to its point of bifurcation (at "B", figure 1) using iridectomy scissors. A fine hooked needle was passed through the lesion to check that no fibres remained intact. The pattern of blood vessels lying on the tract was very variable: occasionally some damage to them was inevitable, but bleeding was seldom fatal.

A small crystal of Horseradish Peroxidase (HRP) was inserted into the cut as soon as possible after the lesion was made. Crystalline HRP (Sigma type IV or Boehringer Mannheim GmbH, grade I) was prepared in advance by allowing evaporation of water from a saturated aqueous solution of the enzyme. Each crystal used was shaped to match approximately the cross-section of the tract: the size of the crystal inserted was of the order of  $400 \times 500 \times 50 \mu\text{m}$ . On wetting the crystal during insertion it became a gel and adhered to the cut surfaces, where it gradually dissolved.

The tissue paper was removed and the left forebrain lobe restored to

its original position before the bone plate was replaced in the cranium.

This operation was performed on the fish described in the first experiment (Chapter 3), labelling axons in a normal optic tract and in tracts formed during various periods of regeneration after mid-orbital transection of the nerve.

## 2.5 PUNCTATE RETINAL LESIONS; APPLICATION OF HRP

Sclera close to the optic nerve head was exposed (on any selected side) by everting the right eye and tearing the conjunctiva. A sharp mounted needle was pushed through the sclera about 500  $\mu$ m from the edge of the optic nerve, puncturing the subjacent neural retina and releasing humor from the eye. The hole in the retina was plugged without delay with a small fragment of "gel - foam" soaked with a concentrated solution of HRP, except when a blood vessel on the retina was inadvertently damaged: when blood was seen to flow from the lesion the fish was discarded. No attempt was made to seal the hole in the sclera, but care was taken to avoid compression of the eye while returning it to the orbit, so as not to eject the HRP from the retina.

## 2.6 POST - OPERATIVE SURVIVAL

It was established empirically that enzyme applied in the retina could be detected in optic axons in the tectum not less than 18 hours later. An interval of at least 48 hours was allowed for HRP applied to the optic tract to reach peripheral retina. Standard transport times of 24 hours after retinal applications and 5 to 5.5 days after tract applications were usually allowed before the sacrifice of the fish.

Survival after punctate retinal lesions was 24 hours.

## 2.7 PERFUSION AND FIXATION OF FISH

Protocol A was used for fish in which HRP had been applied to the optic tract; B was used after retinal application.

## Protocol A

Each fish was kept in darkness for the last hour before perfusion and during induction of anaesthesia; during perfusion it was hooded with aluminium foil to prevent light falling on the retina. These precautions facilitated subsequent separation of the neural retina from the retinal pigment epithelium.

The heart was exposed by removal of part of the ventral body wall, and the fish perfused through the conus arteriosus with 10 ml 0.9% saline. This cleared retinal blood vessels of red corpuscles, which have an intense HRP - like activity on diaminobenzidine (DAB) and would otherwise have obscured the distribution of HRP - filled ganglion cells.

After perfusion the flat antero - lateral wall and the lens of the right eye were removed by circum\_cision of the sclera at the limbus. A radial cut was made in the ventral pole of the retina, adjacent to the vestigial scar in the sclera marking the former position of the ventral fissure. This ensured that subsequent orientation of the retina could be correct.

Gentle irrigation with 0.9% saline ejected from a hypodermic syringe with a narrow bore needle flushed out the humor and then lifted the retina off the pigment layer. The retina was freed by cutting the optic nerve head, and transferred to 0.9% saline where any remaining fragments of the pigment layer were removed with force<sup>s</sup> or by gentle brushing. Jelly coating the vitread surface of the retina was also peeled off. The ventral cut was extended to the optic nerve head and incomplete radial cuts made from dorsal, nasal and temporal poles. The retina was then flattened out and allowed to dry for about 15 minutes at 24 to 30 C on to a gelatinized slide, vitread side up. It was then fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.4) for 30 minutes at room temperature.

Immediately after removal of the retina the fish was decapitated and

the head immersed in more of the same fixative. During fixation the brain was dissected out with the optic nerves attached, by cutting the sphenoid cartilage, tenacula, rhombencephalon and other cranial nerves; the telencephalon was also removed. A tag of sclera was retained on the nasal side of the optic nerve head to assist subsequent orientation. The brain and optic pathways were fixed for 12 - 24 hours in 3% glutaraldehyde in buffer.

#### Protocol B

After dark - adaptation, saline perfusion and circumscision of the sclera (as above) the vitreous humor was flushed out with 3% glutaraldehyde in phosphate buffer (pH = 7.4), which was also used to separate neural and pigment layers. The retina was freed and cleaned briefly in saline as described above, and flattened between glass slide and cover-slip whilst immersed in fresh fixative for 15 to 30 minutes.

Following removal of the retina the rest of the fish was re-perfused with 20 ml of this fixative, or decapitated and immersed in fixative as above. During dissection in fixative the two optic tecta were separated from each other, but the continuity of each optic pathway was preserved.

#### 2.8 PREPARATION OF HISTOLOGICAL SECTIONS

Specimens of brain (including optic pathways) were washed in phosphate buffer (pH = 7.4) for 20 minutes and embedded in a supporting medium of 30% egg albumin and 3% gelatin in water (Grover and Sharma, 1981). Optic pathways from protocol B were gently straightened at the time of embedding. This medium was cooled (4 C, 30 - 60 minutes) until sufficiently solid for a block containing the specimen to be cut from it and transferred to more fixative for "curing" (12 to 24 hours, room temperature).

The block was then infiltrated with 30% sucrose in 0.1 M phosphate buffer (pH = 7.4, room temperature) for twice the time taken for it to

sink (total 3 - 4 hours). This block was shaped asymmetrically to permit subsequent orientation of sections, which were cut at 40  $\mu$ m on a "Pelcool" freezing microtome. The rate of freezing was increased by heaping powdered dry ice over the block on the microtome stage: this was found to reduce morphological damage presumed to be caused by ice crystals. Coronal sections were cut from specimens prepared according to protocol A; specimens from protocol B were cut orthogonal to the optic nerve and tract.

Sections were transferred to more phosphate buffer prior to histochemistry.

### 3. HORSE RADISH PEROXIDASE HISTOCHEMISTRY

Protocol 1 was used after application of HRP to the optic tract; protocol 2 after application to the retina. Retinas were processed either unmounted, in 5 ml specimen jars, or mounted on gelatinized slides in petri dishes. Sections were processed in individual cavities of a perspex tray.

#### Protocol 1:

- 1) Wash in 0.1 M phosphate buffer, pH = 7.4, 2 - 24 hours.
- 2) 0.87 M sodium cacodylate / HCl buffer, pH = 5.8, 30 - 60 mins.
- 3) 1% Cobalt chloride in cacodylate buffer: 20 minutes.
- 4) Wash in cacodylate buffer, 20 minutes.
- 5) "Presoak" in 50 mg% DAB in cacodylate buffer: 30 mins. for retinae; 10 mins. for sections.
- 6) Add 4 drops of 6% hydrogen peroxide per 20 ml for retinae, or 9 drops per 120 ml for sections, and mix.
- 7) Allow reaction to proceed for 20 - 30 minutes for retinae, 10 minutes for sections.
- 8) Wash twice in cacodylate buffer, 5 - 10 minutes each.

9) Counterstain retinae with cresyl violet (Drury and Wallington, 1967).

10) Dehydrate in a graded ethanol series; clear in xylene; mount in XAM.

#### Protocol 2:

1) 0.1 M phosphate buffer, pH = 7.4, 2 - 24 hours.

2) 0.87 M sodium cacodylate / HCl buffer, pH = 5.8, 30 minutes.

3) "Presoak" in a solution comprising:

50 mg% DAB

50 mg% cobalt chloride

50 mg% ammonium nickel sulphate

in cacodylate buffer, 30 minutes.

4) Add 1 drop 6% hydrogen peroxide per 100 ml.

5) Allow reaction to proceed for about 10 to 20 minutes.

6) Wash thrice in cacodylate buffer, 5 mins. each.

7) Dehydrate, clear and mount as in protocol 1, but without counter - staining.

#### NOTES ON HRP HISTOCHEMISTRY

a) All reactions were performed at room temperature, 18 - 22 C

b) Concentrations of solutions were accurate to within 1%.

c) Buffers were accurate to within 0.1 pH unit.

d) DAB = 3,3' diaminobenzidine tetrachloride, supplied by BDH or Sigma chemical company. This was stored below 0 C with a desiccant and solutions were filtered before use. DAB is a presumptive carcinogen (Adams, 1977), and was accordingly handled with care and denatured with bleach before disposal.

e) Grade 1 HRP was used to maximise enzyme activity and histochemical sensitivity.



f) Choice of fixation method is dictated by the need to preserve HRP function while simultaneously inactivating catabolic enzymes. Prolonged fixation has a deleterious effect on HRP activity (Rosene and Mesulam, 1978). Adams (1977) recommended exclusive use of glutaraldehyde, of which Malmgren and Olsson (1978) advocate a dilute (1.25% v/v) solution. This was found to be inadequate for morphological preservation in studies preparing for the present work. A recent alternative is the histochemical development of HRP prior to tissue fixation (Fujisawa et al., 1981a). Trial imitation of this procedure for the present investigations was not rewarded by improved results.

g) Change from protocol 1 to 2 was prompted by discovery that incorporation of Nickel Ammonium Sulphate improves the intensity of DAB reaction product (Adams, 1981).

h) Ideal histochemistry would combine very high sensitivity (characteristic of the chromogen Tetramethylbenzidine (TMB): Mesulam, 1978) for detecting small quantities of HRP in axons and cell bodies remote from the site of application, with good morphological definition of the axons. In a preparatory comparison of alternative histochemical methods, TMB did appear to be more sensitive than DAB; but its reaction product was coarsely granular and morphologically identical regardless of whether HRP-filled axons were present, or whether background precipitation of pigment had occurred spontaneously without enzymic catalysis. This would be a great obstacle to the identification of HRP-labelled axons where these are scattered in the optic pathway. In contrast, the DAB reaction product fills the axon, whose characteristic shape is therefore visible and an aid to identification.

#### 4. MICROSCOPY AND DRAWING OF HRP - LABELLED RETINAE.

Reichert and Leitz microscopes were used. High magnification and resolution were necessary for the identification of HRP - labelled ganglion cells after application of the enzyme to axons in the optic

tract. To obtain an accurate montage of the distribution of these cells in the whole retina a montage was compiled from drawings of small areas. Each of these was made with the aid of an eye-piece graticule, by marking the positions of labelled cells and of the edge of the retina on to corresponding positions on graph paper. The montage of all the drawings was traced and photographed.

Photomicrographs of histological material were taken on a Leitz microscope using Ilford Pan F film.

## 5. COUNTING CELLS

Retinal ganglion cells stained with cresyl violet could be distinguished from other cells present by their morphological characteristics, positive identification of the "type" being provided by those also stained with the brown reaction product of HRP / DAB histochemistry.

A simple sampling procedure was used to gather information about the population density of ganglion cells in different annuli of the retina. The radius was divided into about nine units each equivalent to the width of one field of view in the microscope, using the x40 objective. The cells present within one field of view were counted for each position along four radii. For each annulus the average of these samples was used as an index of population density.

## 6. VISUOTECTAL MAPPING

After induction of anaesthesia mucus was wiped off the body of the fish, which was then wrapped in aluminium foil lined with wet tissue paper: this ensured good electrical contact with minimum generation of electrical "noise". The fish was positioned, and kept anaesthetised, as above. The octagonal bone in the cranium was removed and fluid on the surface of the brain was absorbed with tissue paper without damaging the

superficial blood supply of the tectum. The brain was covered with light paraffin B.P. Wet tissue paper was placed over the mouth to prevent drying and post-operative rigidity. The cornea was moistened intermittently without moving the eye.

Thus prepared, the fish was manoeuvred in perimetry apparatus (Aimark Co., modified) so that the right eye lay at the centre of a hemisphere defined by a rotatable arc of the apparatus (radius 33 cm); and oriented such that the visual axis was co-axial with the rotation of the perimeter arc. For this purpose the visual axis of the eye was defined as that along which the optic nerve head is visible when trans-illuminated by a light behind the fish - the "papillary axis". There is apparently no macula in the goldfish.

The orientation of the eye was estimated from the position of the vestige of the ventral fissure, marked on the sclera. This was checked at intervals during mapping to ensure that the eye had remained immobile. Visuotectal maps are drawn as though this mark had been ventral.

A micro - electrode was supported in a calibrated micro - manipulator and could be inserted into the dorsal part of the tectum along its own axis, approximately perpendicular to the dorsal - most part of the tectal surface. This electrode was used both for recording visually - evoked electrical activity, and as an inert probe to obtain two - dimensional coordinates for points along the perimeter of the accessible part of the tectum: recording positions are identified with reference to this outline, which delineates the horizontal planar projection of the curved tectal surface.

Uninsulated sharp tungsten needles with a tip diameter of less than  $1\text{ }\mu\text{m}$  (tapering from  $200\text{ }\mu\text{m}$  over about 2 mm) were used to record visually - evoked activity for most of the maps presented here. Insulated electrodes (tungsten in glass: Merrill and Ainsworth, 1972; and tungsten in varnish) were also used: these were judged subjectively (by listening

to an audible output of recorded signals) to be no more sensitive, and to indicate similar receptive field sizes.

Electrical signals picked up by the electrode were fed through a field effect transistor and an audio amplifier for acoustic identification. Greatest clarity was obtained with a gain of 10,000x, and with filters excluding input frequencies below 300 Hz and above 1000 Hz. The reference electrode was connected to the fish and to the metal tubes through which anaesthetic entered and left the fish's perfusion bath. All metal in the micro - manipulator and perimeter was earthed.

At each recording site the electrode tip was inserted to a depth of approximately 150-200 $\mu$ m. This depth was adjusted to maximise the amplitude of responses.

The visual stimulus used was a D.C. light source (2.5 W, 9 V) subtending 2° at 33 cm. The sides of the stimulus were encased in opaque material to avoid inadvertent shadows and reflections. The background was dark. The clarity of responses could be maximised by turning the stimulus on and off. No attempt was made to identify optic axons with particular response characteristics.

Only multi - unit responses were elicited using uninsulated electrodes. With insulated electrodes single - unit responses were occasionally obtained at some electrode depths, but these were not used in the visuotectal maps.

At each recording site the multi - unit receptive field size was estimated, and the optimum stimulus position (corresponding approximately to the centre of the multi - unit receptive field) was recorded. Care was taken to search the entire visual field for additional stimulus positions: where two local optima were present, both were recorded. Except for noting unusually weak responses, their amplitude was not quantified.

Stimulus positions were measured using the perimeter frame, in

coordinates which identify (i) the orientation of the plane containing both the axis of optic centring and the stimulus position, relative to the horizontal plane through the nose (the "circumferential coordinate"), and (ii) the angle within that plane between the centred (papillary) axis and an imaginary line from the optic nerve head along the stimulus direction (the "radial coordinate"). These coordinates were subsequently transferred to a flat disc - chart whose circumferential coordinate is proportional to the first, and whose radial coordinate is proportional to the second. (Transforming a hemispherical "surface" of possible stimulus directions on to a plane in this way necessarily distorts the representation of solid angles of visual space: this does not matter for present purposes.)

In the visuotectal maps (figures 50 to 78) corresponding recording sites and stimulus positions are denoted by the same number in the map of the tectum as in the map of the visual field. The sizes of these symbols are selected for cartographic clarity and are unrelated to the sizes of the visual fields.

To avoid bias an independent observer was asked to locate optimum stimulus positions (while ignorant of the electrode's location) whenever possible. Some points were mapped out of sequence with their neighbours to reduce biased anticipation of the stimulus direction. There remains a subjective element in the mapping of only faintly audible responses.

After mapping, the paraffin was floated off the surface of the brain on drops of saline; the bone plate was replaced in the cranium; and the fish allowed to recover from the anaesthetic.

Attempts to keep the fish alive for more than a few days after mapping were unsuccessful, except in normal fish mapped only briefly and returned to saline afterwards.

## CHAPTER THREE

### RESULTS OF RETROGRADE LABELLING OF REGENERATING FIBRES

Figures 2 to 17 show flat-mounted right retinae (ventral at the bottom, nasal to the right; the optic papilla is indicated by the central ring). Dots indicate the positions of ganglion cell bodies labelled with HRP following application of the enzyme to a cut through the whole cross section of the regenerating optic tract. The duration of regeneration prior to this operation is shown by the number of days below each retina.

Labelled ganglion cells were identified by the following criteria. In some cases both the cell bodies and a few micrometers of the axons emerging from them are well-defined by the characteristically speckled brown HRP reaction product, clearly visible against the Cresyl Violet stain ( Plate 1). Because these cells all lie in a single layer, alteration of the plane of focus of the microscope allowed simple differentiation between these cells and occasional flecks of pigment epithelium adherent to the opposite surface of the neural retina, or foreign bodies lying on the vitreous surface - both of which, seen out of focus, can have a deceptive appearance. Ganglion cells are distributed in radiating rows within this lamina, but these merge together at the periphery of the retina. The only other cells in the retina which stain with Diaminobenzidine are the endothelial cells of the blood vessels and erythrocytes not flushed out by perfusion: these have a characteristic and contrasted morphology and pattern of staining. Other ganglion cells in which the proximal part of the axon was not seen were identified by the similarity of their size, shape and distribution as compared with the former group. The distribution of the cells has been drawn, not photographed because it is impossible to photograph with sufficient resolution without making a huge montage of the retina, and because of

the need to alter the plane of focus whilst identifying cells.

The figures show the results from fifteen fish in whom the optic nerve has been cut, together with a normal fish (figure 17) labelled in the same way but without prior nerve transection.

The retinas in figures 2 to 12 comprise a series: their optic nerves were cut on the same day, they were kept in identical conditions, and they were labelled after increasing intervals. In figure 2 very few of the ganglion cells have been labelled, and they are distributed apparently without central clustering. In figure 3 the number of cells labelled is also small, but they are clearly grouped around the centre of the retina. With the exception of figure 2, successive figures show that with increasing duration of regeneration the cells remain distributed as a central group, but the radius of this group increases until it reaches the perimeter of the retina - as in the fish labelled after 48 and 84 days of regeneration (figures 11 and 12).

Figures 13 to 16 show results of a similar experiment performed subsequently. (Repetition was made imperative by a report of results contradicting those described above (Cook, personal communication), discussed below.) These figures also show a central group of labelled ganglion cells, and are compatible with the trend shown more clearly in the preceding series. However, the retina shown in figure 14 contains an arc of 20 very densely labelled ganglion cells round part of the edge of the group which has been drawn. These may be cells whose axons were uncut in the initial nerve transection; and interpretation of the fainter labelling of other central cells in this retina is therefore precluded.

In the same series of fish two retinae were devoid of HRP (applied after 18 and 27 days of regeneration), and six contained labelled ganglion cells which were scattered throughout the retina, not confined to a central group. This pattern was observed after 16, 16, 21, 24, 24 and 27 days of regeneration: fewer than 30 cells were labelled in each of

the first three. The morphology of HRP-staining is the same as in the retinae with only central labelling. After longer periods of regeneration (e.g. 27 days) the HRP/DAB staining is denser (plate 2).

Sixteen retinae taken from fish in the first series in which the interval between optic nerve cut and application of HRP was less than 22 days were found to contain no labelled ganglion cells.

Figures 18 to 24 show graphs of the population density of ganglion cells (identified after staining with cresyl violet, those also stained with HRP/DAB being used to define the "type"), plotted against the radius of the retina for some of these specimens. The duration of regeneration is indicated under each graph. Similar data from three normal retinae are presented for comparison (figures 25 to 27). In each retina cresyl violet - stained ganglion cells were counted along four radii, shown by separate lines on the graphs. There is marked fluctuation of cell density over small distances, but no consistent asymmetry of distribution. After optic nerve cut the retinae contain fewer ganglion cells than normal. There is no obvious increase in the number of cells with increasing duration of regeneration.



DISCUSSION OF RETROGRADE LABELLING OF REGENERATING FIBRES1) Choice of method

The purpose of the experiment is to display the sequence in which axons arrive at the tectum during regeneration. No anatomical method allows repeated assay of successive stages of regeneration in the same fish. Electrophysiological mapping could possibly provide a means of achieving this, but on the occasions it has been attempted in investigation of successive stages of development (Gaze and Jacobson, 1963; Gaze and Keating, 1970, both using *Xenopus*) very few animals survived long enough to be re-mapped. Whether the sequence is investigated in one animal or inferred from a group, the limitations intrinsic to electrophysiological mapping complicate the interpretation of results. Firstly, there is no guarantee that visually evoked electrical activity is present in regenerating fibres ab initio, so the report of topographic order in the first detectable responses from a regenerated projection (Horder, 1971b) cannot be taken as firm evidence of synchrony in the reformation of synapses. As a hypothetical example, if axotomy causes papilloedema, this could result in temporary alteration of impulse conduction lasting until the projection had reformed. Secondly, although it is well established that most micro-electrodes detect pre-synaptic activity, and that this is probably at branch points in optic fibre arborizations close to their terminations (Maturana et al., 1960; Cronly-Dillon, 1968; Gaze, 1970; George and Marks, 1974) this applies to recordings made from mature projections. There is no evidence about the sources of recorded electrical activity in early stages of regeneration. Impulses in growth cones and unmyelinated fibres of passage may contribute to the appearance of disorder in some maps of newly-

regenerated projections (Schmidt and Edwards, 1983). Thirdly, even detailed mapping by standard methods affords no guarantee that small numbers of fibres will necessarily be detected. The newer method introduced by Freeman (1977) using current source density analysis of signals in several electrodes used simultaneously may offer some improvement in this respect, but has not been extensively tried.

For these reasons it is necessary to resort to anatomical methods. These offer greater resolution: instead of inferring the approximate positions of ganglion cells from their receptive fields in visual space, one may identify individual cells histologically. Sacrifice of the animals imposes the need to compile a composite picture of the sequence of regeneration from a time series involving several fish.

Retinotopic identification of ganglion cells whose axons have reached the tectum (after any selected period of regeneration) cannot readily be achieved by direct tracing of the axons through the optic pathway and into the retina. To distinguish newly-regenerated, fine, unmyelinated axons (Horder, 1974b) from the debris of previous fibres either a selective histological stain or electron microscopy would be necessary. Attardi and Sperry (1963) used a modified Bodian - Protargol method which stained new axons in a distinctive manner: perhaps the axons' appearance after staining with silver is affected by the influx of cytoskeletal proteins which occurs during their maturation (Murray, 1976; Hoffman and Lasek, 1980). Unfortunately this method has proved impossible to repeat. Serial electron microscopy would be extremely laborious and, in the optic nerve head, very difficult.

As an alternative, numerous methods of tracing axons have been devised which locate cells, axons or terminals. In many instances a neuron may be identified by a "chromatolytic" reaction in the cell body induced by damaging the axon (Cragg, 1970). This would not be a suitable marker in this case: classical chromatolysis is not seen in goldfish

neurons, although there is an increase in the density of Nissl substance (Murray and Grafstein, 1969); and cells showing this response after a second surgical lesion (damaging only those axons which had regenerated) might be indistinguishable from those still reacting to the first axotomy, at optic nerve transection: responses to axotomy are very variable in time and extent (LaVail, 1975).

The various exogenous compounds used in axon tracing have specific uses and limitations. Use of radio-labelled amino acids is restricted to anterograde tracing from the perikaryon (e.g. Meyer, 1980). Cobalt has proved useful for intra-cellular injection and axon filling over short distances (e.g. Tyrer and Altman, 1974), and for anterograde tracing of groups of axons (e.g. Steedman, Stirling and Gaze, 1979). But as a means of labelling a small number of axons for retrograde transport over long distances, Cobalt is unsuitable: this is partly because of its extensive diffusion from the site of application. Certain fluorescent dyes are also used for retrograde tracing of axons (e.g. D.A.P.I. (Yeziarski and Bowker, 1981), Rhodamine B Isothiocyanate (R.I.T.C.) (Thanos and Bonhoeffer, 1983)).

Horseradish Peroxidase (HRP), however, has been most extensively used for retrograde and anterograde tracing (LaVail, 1975), and was adopted for these studies.

## 2) Choice of experimental procedure

In this experiment it is necessary that HRP should meet the following criteria. It should:

- (i) label axons which have regenerated to the tectum;
- (ii) not diffuse extensively, and thus not be taken up by axons not selected by axotomy;
- (iii) enter all the cut axons at its application site;
- (iv) be transported retrogradely to the ganglion cell bodies up to about 5 mm distant;

- (v) neither diffuse nor be transported between ganglion cells;
- (vi) retain its activity throughout transport and subsequent histological processing; and
- (vii) identify labelled ganglion cells distinctively from unlabelled cells.

Some of these requirements are reflected in the experimental method.

(i) In order to label ganglion cells whose axons have reached the tectum, the ideal site of enzyme application would be the tectum itself. However, ventral tectum is not readily accessible, and because of the size of the tectum and the diverse routes taken by regenerating axons (Udin, 1978; Cook, 1983), it would be impossible to guarantee that all axons have access to HRP except by application of a large volume of enzyme. Diffusion of this towards the eye could result in the labelling of uncut axons in the optic tract as well: growth cones are able to take up HRP (at least when neurites are grown in culture: Bunge, 1977).

To avoid the problems inherent in diffuse application of HRP, axons were cut in the regenerating optic tract close to its division into two brachia, and enzyme applied to this restricted area. It is possible, though unlikely, that the sequence of arrival at this point differs from the sequence of arrival at the tectum, which is about 300  $\mu\text{m}$  further caudal.

(ii) The use of crystalline HRP favours a more restricted distribution of enzyme at its application site. Sections of the optic tract and brain obtained 5 days after enzyme application reveal HRP at the site of the tract lesion but extending not more than 80  $\mu\text{m}$  rostrally.

(iii) The stipulation that all axons which had grown as far as the tract lesion should take up HRP requires that the lesion should be complete, and that the HRP should be present across the entire cut surface. At operation the completeness of the lesion was confirmed by passing a

needle through it. If any axons were accidentally spared, this would probably not have altered the pattern of retinal labelling: axons regenerating in the optic tract after the optic nerve has been cut generally show no spatial orderliness according to the radial coordinates of their retinal origins (Dawnay, 1981a). Even supposing that there is some residual topography in the tract, any cut through deeper (medial) axons necessarily also cuts the axons of peripheral (unlabelled) ganglion cells because these lie superficially (Dawnay, 1979a): partial lesions should label peripheral cells at least. It was also obvious at the time of surgery that the HRP contacted peripheral as well as deep fibres.

A separate and important point is that this method of identifying cells whose axons have regenerated is valid only if there is no radially retinotopic difference in the ability of these fibres to take up and transport the enzyme. HRP can enter injured mammalian axons by passive diffusion (Kristensson and Olsson, 1976); and there is no evidence that any other, selective mechanism is necessary in goldfish. The ability of axons to transport HRP while they are still growing has not been well investigated. Olsson, Forsberg and Kristensson (1978) reported that there was very little retrograde axonal transport of HRP between axotomy and reformation of neuromuscular connections in the facial motor neurons of the mouse. But in that study HRP was administered by intravenous injection, and the limiting factor may have been not the axoplasmic transport, but the access of the enzyme to the axon: HRP leaked into the nerve at the site where the axons were crushed, but during regeneration the growth cones became further removed from this, and little HRP enters axons through their plasmalemma (Bunge, 1977).

There is no reason to anticipate that uptake and retrograde axoplasmic transport of HRP depend on some attribute which has a radially retinotopic distribution in fibres which regenerated synchronously.

(iv) The ability of goldfish optic axons to transport HRP over their

entire length has been validated experimentally (Dawnay, 1981 b).

(v) No transfer of HRP between ganglion cells has been reported; and this enzyme's ability to label isolated ganglion cells is demonstrated in these results.

(vi) The half-life of HRP activity in goldfish ganglion cells has not been measured, but in experimental trials it was found that the HRP-catalysed reaction with DAB could still be produced 8 days after application of the enzyme. In chick retinal ganglion cells HRP has been found to last only 3 - 4 days (LaVail and LaVail, 1974): this difference may reflect faster catabolism in the warmer animal.

(vii) Histochemical processing with DAB of retinas and sections of the optic pathway lacking HRP confirmed that ganglion cells possess no endogenous HRP-like enzymes. A dense reaction product was deposited in red blood corpuscles and a scanty reaction product in the endothelial cells of capillaries. The morphology of these cells makes them readily distinguishable, but because dense labelling of blood might conceal underlying ganglion cells, it was necessary to perfuse the retinas before fixation.

### 3) Interpretation of the results

With the exception of figure 2, drawn specimens of the first series labelled after brief periods of regeneration show that only central ganglion cells are labelled. Several possible explanations for this must be considered. For reasons already discussed, failure to label peripheral cells is not attributable to denying their axons access to HRP; and there are no grounds for supposing that there is retinotopic variation in the speed with which axons re-acquire the ability to transport the enzyme, unrelated to the sequence of regeneration. A third explanation is that insufficient time was allowed for carriage of HRP to the periphery of the retina early in regeneration; but varying the interval between

application of HRP and the sacrifice of the fish between 2 and 8 days produced no consistent increase in the radius of the group of labelled cells.

The most likely explanation for the observed patterns of labelling is that ganglion cell bodies are labelled when their axons are labelled, and not when their axons are not. In particular, absence of HRP in a ganglion cell may be taken to indicate that its axon was not labelled. If this is so, the set of figures indicates a central - to - peripheral sequence in the regeneration of axons to the site of application of the enzyme. There is no reason to expect the sequence of their arrival at the tectum (close behind) to be grossly different.

The only studies of the sequence of regeneration previously published are those of Gaze and Jacobson (1963) and Gaze and Keating (1970). In these experiments visuotectal mapping was used to demonstrate the extent of regeneration after different periods

following optic nerve transection in *Xenopus*. The limitations of this method have been noted above. Gaze and Jacobson (1963) noted that after brief periods the recorded electrical activity was abnormal in being inconstantly elicited; in fatiguing fast; and in showing enlarged multi-unit receptive fields (up to  $50^\circ$ ). Some examples did show tectal representation of only central visual field, indicating a projection from central retina; but others showed peripheral field maps only. The correct interpretation of these partial maps is not certain. Four distinct patterns of visuotectal map were found. In pattern 1, only small parts of the visual field were represented on the tectum, and there was little or no apparent order. In pattern 2 also, only part of the visual field was shown to be represented, but some semblance of retinotopy could be discerned among some of the mapped loci. (The example depicted in Gaze and Keating (1970) is approximately topographic in the medio-lateral axis of the tectum, but not in the rostro-caudal.) Pattern

3 was a normal visuotectal projection; and pattern 4 had the appearance of a normal map with the addition of a contralateral version of the partial map normally found on the ipsilateral tectum in this species.

The authors suggested that patterns 1, 2, and 3 or 4 represent successive stages in the regeneration of the projection: one example showing a pattern 1 map subsequently produced a normal map. However, examples of pattern 1 also occur after relatively long periods of regeneration: the fourteen examples presented by Gaze and Jacobson (1963) were obtained at: 23, 29, 31, 33, 36, 40, 43, 51, 68, 68, 69, 72, 91, and 103 days, most of these being periods which under usual conditions would result in the formation of normal maps. This span also overlaps considerably the periods of regeneration resulting in patterns 3 or 4: e.g. pattern 3 after: 33, 35, 45, 47, 48, 56, 60, 65, 68, 113, 125, and 200 days. It is therefore not clear how these maps relate to the anatomical study of the sequence of regeneration presented here.

#### 4) Precision of retinotopy in the sequence of regeneration

Some of the retinas show a sharp boundary to the group of labelled cells, but in others this cut-off is relatively imprecise. However, this does not mean that the sequence of regeneration must also be imprecise: if the ends of growing axons are able to take up this enzyme (Bunge, 1977), diffusion of HRP further down one side of the tract than the other would produce exactly this effect. Alternatively, the population of axons may show a normal distribution in the quantity of enzyme which they are able to take up and transport: as the concentration of diffused HRP diminishes with increasing distance from the site of the lesion a smaller proportion of axons will take up and transport enough enzyme to exceed some hypothetical threshold for detection. (It is not clear why the boundary between labelled and unlabelled areas should be sharper in some fish than in others).

For these reasons the present experiments cannot be used to



quantify the precision of retinotopy in the sequence of regeneration. It must be pointed out, however, that in the absence of a method for quantifying the precision of topography in the radial dimension of the synaptic array after regeneration, there is no objective means of knowing how accurately retinotopic the sequence of arrival would need to be to fulfil the postulated organising role.

#### 5) Errors of retinotopy in the sequence of regeneration

If new ganglion cells are generated at the perimeter of the retina during regeneration, their axons will arrive at the tectum in consort with regenerating axons from more central annuli. To this extent the sequence of arrival would not be retinotopic. The importance of this is diminished if, as in this experiment, the fish remain at approximately constant length during regeneration; but this is one possible explanation for the labelling of a few peripheral cells in figure 13. Since the growth of the retina is slow compared with the regeneration of axons, the number of new axons is likely to be so small (under any circumstances) that any consequent error of topography could easily be undetected by conventional visuotectal mapping.

A second problem arises from the observation that after short periods of regeneration the population density of labelled cells within the central group may be much lower than in a similar area of a retina obtained after a longer period of regeneration (compare the retinas in figures 4 and 11, for example). But comparison of different fish is unreliable on two counts. Firstly, the population density of labelled cells would be reduced if an application of HRP were accidentally inadequate to allow all fibres present to take up the enzyme. Theoretically, this could occur because of incomplete transection of the tract, or application of too small a crystal of HRP; but operative technique was intended to avoid these, and no evidence that either

occurred was seen at the time of the operation, or during subsequent histology of the tract. Secondly, it may be that in a population of cut axons only a proportion will take up sufficient enzyme to be detected. If this uptake is related to the cross-sectional area of the axons, which is likely to be normally distributed within the population, and which increases with increasing duration of regeneration (Murray, 1976), then one would predict precisely this result: that a small proportion of the axons present will be labelled <sup>sufficiently</sup> after brief periods of regeneration, and a larger proportion after long. The results presented therefore cannot be taken as evidence that any ganglion cells regenerate late, out of the radial sequence, although this cannot be excluded with certainty either.

The estimations of ganglion cell population density obtained by counting samples of cresyl violet-stained cells (page 52) show that there is no major formation of new cells during the period of regeneration (figures 18 to 24). This conclusion agrees with that of Murray and Forman (1971). Labelling with tritiated thymidine has shown the absence of nucleic acid synthesis in ganglion cells of *Xenopus* during regeneration (Bohn and Reier, 1982). This potential cause of alteration in the sequence of axonal arrival at the tectum is therefore unlikely. (The data presented here (figures 18 to 24) have only a specific use in excluding dramatic changes in the number of ganglion cells in these particular fish: because the shrinkage of the retinas during histological fixation was not well controlled the measured population densities are not accurate indicators of the true values).

Another possible cause of disturbance to the centrifugal sequence of axon arrival must also be considered. If some cells enter a relatively prolonged recuperative phase after axotomy, their axons will regenerate after those of their neighbours. Staining with cresyl violet did not indicate such variation between cells, but is not necessarily the ideal method with which to look.

One must conclude that the evidence from these figures of a central-to-peripheral sequence of labelling in the retina is not adequate to conclude that all the ganglion cells conform to this sequence of regeneration. If it should subsequently be shown that some axons do arrive at the tectum later than others with the same retinal radial coordinate, any contribution they make to the topography of the map must be achieved by a different mechanism. It could be, for example, that late-regenerating axons might be led to their termination sites by "contact guidance" along their predecessors (cf. Horder and Martin, 1978).

#### 6) The significance of these results

The single major problem in evaluating these results is that they are not completely reproducible. In the second series the majority of fish with HRP - labelled cells (six of ten) showed that labelling was not restricted to central retina; and in the first series this is seen in figure 2. In unpublished experiments of a similar nature J. E. Cook and C. A. O. Stuermer have each obtained retinae with HRP - labelled ganglion cells scattered throughout their radial extent (personal communications). Cook applied 30% HRP solution on gel-foam to a cut across dorsal rostral tectum after 21 to 50 days of regeneration following mid-orbital cut through the optic nerve: one fish (21 days) contained no labelled cells, five (21, 29, 35, 42 and 50 days) contained a large number of cells (not quantified) distributed apparently uniformly over the corresponding sector of retina.

Those of Stuermer's fish with HRP in the retina included three with only central labelling. Few cells were marked in those obtained after 9 and 11 days of regeneration. After 14 days the pattern closely resembles that shown in figure 4. After similar periods of regeneration (13 and 14 days) two showed unlocalized labelling. Sixteen contained many ganglion

cells with HRP, distributed all over the retina, after longer periods of regeneration (15 days to 2 months); these resemble figures 11 and 12.

It may be possible (but not necessarily correct) to explain these workers' contradictory results as artefacts of experimental technique: for example, there could have been faster regeneration because of higher temperature (Springer and Agranoff, 1977) or other seasonal factors (Stuermer and Easter, 1984), coupled with labelling of only a random subset of the axons present. Or there could have been an excess of HRP which by diffusing towards the eye might gain access to axons not yet arrived at the application site. But excuses of this type cannot be adequate to account for the conflict among the results presented here, of experiments performed by one person with one technique at one time. It is currently an open question whether the unrestricted or the centrally restricted pattern of labelling is an artefact, or whether there is genuine variation in the sequence of axon regeneration, so that it is sometimes retinotopic and sometimes not.

If the experiment is repeated, some clarification may be obtained by using the new axonal marker Rhodamine B Isothiocyanate (Thanos and Bonhoeffer, 1983) in measured amounts in place of HRP: its low solubility in water may reduce diffusion and allow very restricted application sites.

If there is a retinotopically specific sequence of axonal arrival, its significance can be assessed only by experimental intervention. If retinotopically specific alteration of this sequence correspondingly alters the radial topography of the retinotectal projection, this will confirm the importance of time in generating the spatial pattern of connections. This is the reason for the experiment described in chapter 7, in which the regeneration of axons from central retina is delayed relative to that of axons of central origin by cutting them again, a varied interval after initial transection of the entire optic nerve, and the resulting projections are mapped electrophysiologically.

## SUMMARY OF FIRST EXPERIMENT

Central retinal labelling by HRP applied to the regenerating optic tract indicates a central to peripheral sequence of optic fibre regeneration after nerve transection; but this finding is not wholly reproducible.

Examination of experimental techniques might clarify the discrepancy between results.

The experiment outlined in the final paragraph of page 64, and described in chapters 7 and 8 tests the significance of retinotopically sequential regeneration as a possible means of generating order amongst optic terminals.

## CHAPTER FIVE

### METHOD OF ANALYSIS OF VISUOTECTAL MAPS

(with Appendices: A, B, C, D, E)

The representation of the visual field on the tectum has been mapped by recording visually - evoked electrical activity at a set of tectal loci, and identifying for each locus the one or two directions in which a standard light source evokes a locally maximal response. The method used to obtain these maps has been developed by other workers (Gaze, 1958; Maturana et al., 1959; Gaze, 1970); but the method used to analyse them is new. It is therefore important to postpone discussion of specific results until their general format has been described. This chapter explains the method and discusses its limitations. Points are illustrated with maps described in the following chapter.

#### VISUAL FIELD MAPS

One example of a normal visuotectal map is shown in figure 52a. The upper diagram is a representation of the visual field, with the numbers indicating the positions of the optimum stimuli. These numbers do not occupy the ventral-most portion of the visual field because in goldfish this is represented on ventro-lateral tectum and is therefore inaccessible to the recording electrode.

The centre of the circle is the axis of the mapping apparatus. This is also taken to be the optic axis of the eye, because each fish was oriented before mapping so that the transilluminated optic papilla could be seen along this axis: the goldfish lacks any readily identifiable fovea, so only the papilla provides a simple, reliable means of centring.

The optimum visual stimulus positions are defined relative to this central axis by a pair of "polar" coordinates. Termed "radial" and

"circumferential", both of these coordinates are angles whose values are read off the mapping apparatus.

The radial coordinate is measured as the angle between the direction of the stimulus (OS) and the reference axis (OA) (see figure 28). All directions with a radial coordinate "r" are plotted on the diagram as points on the circle whose radius is proportional to "r", and whose centre is the centre of the diagram (figure 30). The perimeter of the diagram is a circle drawn with a radial coordinate of 100 degrees, although the dorsal visual field extends to this limit only in its temporal and uppermost aspects.

The circumferential coordinate is measured as the angle between two planes (illustrated in figure 29), one being the plane OAS containing the papillary axis OA and the stimulus direction OS, and the other being an arbitrary reference plane traditionally (and in this case) that containing the papillary axis and the nasal-most pole of the visual field (CAN). This coordinate therefore has a range from 0 - 360 degrees: any stimulus directly above the papillary axis has a circumferential coordinate of 90 degrees; any directly below, 270 degrees.

On each diagram N marks the nasal pole: points with a circumferential coordinate of zero are drawn on the radius ON. A stimulus direction with a circumferential coordinate "c" is shown on the diagram as lying on the radius OS produced (figure 30).

Because this diagram of the visual field is a planar representation of a spherical surface (defined by unit vectors along the directions of visual space), it is inevitably a distortion. Unit solid angle in visual space is not represented by an equal area in each part of the diagram, and lines that are parallel in space are not shown as parallel on the diagram. Nevertheless these maps do provide a readily comprehended picture which is suitable for certain purposes.

## CARTESIAN COORDINATE TECTAL MAPS

In figure 52a the lower diagram represents the optic tectum by an entirely different convention. The recording electrode positions are drawn as measured: namely, as loci in a two - dimensional Cartesian grid in a horizontal plane (in contrast to the polar geometry used to describe the stimulus positions). The coordinates (g,h) were measured in rostro - caudal and medio - lateral axes respectively. The depth of the electrode tip from this plane was not measured.

Coordinates of recording sites were measured relative to an arbitrary zero, but are plotted relative to the planar projection of the perimeter of the tectum. Within this boundary the planar coordinates of the electrode sites are shown by the positions of numbers. The same number in the upper diagram shows the direction of the optimum visual stimulus for each site. For certain points in some retinotectal projections two local maxima were identified in the visual field ( i.e. two directions in which the visually - evoked response was greater than for neighbouring directions). Both are shown by the same number in the diagram. The relative intensities of the maximum responses are not indicated.

The microelectrode was used as a probe to measure pairs of two-dimensional Cartesian coordinates at six to eight selected positions around the margin of the tectum. These have been used to plot the perimeter line around the map of recording sites (figure 31). The medial boundary is a straight rostro-caudal line. Points on rostral, lateral and caudal extremities of the tectum have been joined up not by a curve of variable radius (cf. Gaze, 1970; Martin, 1978) but by the arc of a circle, which was chosen to fit the points as closely as possible (judged subjectively). This arc is used to estimate the radius of curvature of the tectum, which is used in subsequent computations from the measured data.

Except in this last respect, a similar system of portraying



visuotectal maps has been adopted for many years by other workers. It has the disadvantage of unnecessarily distorting the image of the tectum relative to that of the visual field: the tectum is not a plane surface, it is part of a sphere (to a first approximation: see Appendix A). It ought therefore be portrayed by a geometric projection similar to that used in the upper diagram to represent visual space.

A second problem with the conventional tectal diagram is that it allows little scope for quantifying the orderliness of the visuotectal map. Usually the only test for disorder is to draw two non - crossing lines through adjacent sequences of points on the tectal diagram, and to see if there is any crossing over of the two lines linking corresponding points in the map of the visual field. Gaze and Jacobson (1963, Appendix) devised an application for the Chi-squared test to provide a statistic by which to judge the significance of apparent orderliness of the visuotectal map. This test compares the directions of imaginary lines linking mapped optimum stimuli, with those directions predicted by a very simplified model of a normal map. In a "perfect" map, according to this model, the direction of the line between stimuli is arbitrarily defined as being within 45 degrees of an idealized orientation, which is an approximation to the general orientation subjectively observed in one normal map. The ideal direction on one side of the vertical meridian of the visual field is 45 degrees different to that on the other. This definition of an "idealized" map is self-fulfilling for normal maps, so the method cannot quantify their precision. Nor does this method allow distinction between any two dimensions of topography.

A much simpler method of quantification may be used if either the tectal loci or the visual stimulus directions are re-written, so that a common system of coordinates may be used to describe them both. In a perfect map there will be good correlation between corresponding coordinates, given certain assumptions about the nature of the

topography, which are considered below.

Another potential disadvantage of the traditional tectal map is that it portrays the recording sites in Cartesian coordinates. If mechanisms generating topography really act in radial and circumferential dimensions, it will be more appropriate to consider the accuracy of each map in polar terms.

#### POLAR COORDINATE TECTAL MAPS

All these problems may be solved by manipulating the measured data, so as to be able to re-draw the map of the recording sites using polar coordinates on a spherical model tectum. If these coordinates are defined analogously to those in the map of the visual field, it is possible to calculate the coefficient of correlation between tectal and field coordinates for radial and circumferential dimensions. For this to be achieved, the reference axis for the new system of polar tectal coordinates must be perpendicular to the surface of the tectum at the projection of the optic papillary axis. Although the optic papilla lacks photoreceptors a close approximation to the correct locus can be estimated by mapping a small number of nearby points.

The definitions and computation of the polar coordinates are detailed in appendix B; the computer program is reproduced in appendix E.

The "radial" coordinate is the angle between the tectal radius ending in the recording site (OS') and that ending in the tectal site representing the optic papilla (OA'). It is therefore equivalent to the measured radial coordinate of <sup>the</sup> visual stimulus directions.

The "circumferential" coordinate is the angle between the plane OA'S' and the plane OA'G. OA'S' is the plane containing <sup>the</sup> tectal radii to the points imaging the visual stimulus <sup>OS'</sup> and the papillary axis. <sup>OA'</sup> OA'G is an arbitrary reference plane. It corresponds to the plane OAN in figure 29 if it is assumed that the nasal pole of the visual field is imaged on the

rostral pole of the tectum in a normal retinotectal projection. The maps of normal projections presented here (figures 50a to 55a) and by other workers show that this is at least approximately true; but the distortion inherent in such diagrams makes it impossible to judge how accurately the planes correspond. The effect of any mismatch of the reference planes OAN and OA'G is simply to rotate one diagram relative to the other: the coefficient of correlation between visual and tectal circumferential coordinates will be unaltered.

Appendix C provides a simpler method of calculating polar coordinates on the assumption that the tectum is flat.

The method explained in appendix B has been used to calculate polar coordinates for all the maps. Because radial and circumferential coordinates are defined in such a way as to be compatible with those in the visual field, the same convention may be used to draw the surface of the tectum as is used to portray visual space. This practice has been adopted in re-drawing one example of a tectal map (figure 52d).

The centre of the new diagram represents the tectal image of the papillary axis. The radial coordinate (an angle) is denoted by the distance from the centre to the point representing the recording site, which is indicated, as before, by a number corresponding to a number in the visual field. The circumferential coordinate, also an angle, is shown by the angle in the plane of the diagram between the radius to the recording site and the reference radius to the rostral pole, corresponding to the plane xy (= A'OG) in the spherical model tectum (figure 42). The convention adopted in drawing figure 52d is to produce a mirror image of the tectum so that the reference radius to G lies in the same direction as the radius to "N" in the visual field map, and the circumferential coordinate is measured anticlockwise - again, as in the visual field map. This is simply for ease of comparison of the diagram of the tectum with that of the field.

Comparison of figure 52a with figure 52d (allowing for the mirror - inversion of the revised diagram) shows that the image of the tectum obtained using polar coordinates calculated for a spherical model tectum in this way closely resembles the original tectal map, which was plotted on the false assumption that the tectum is flat.

To assess the importance of treating the tectum as a spherical rather than planar structure, idealized visuotectal maps were created for which the correlation between corresponding coordinates is perfect according to the spherical model (appendix C). Polar coordinates, based on the assumption that the tectum is flat, were also calculated from the same data. Two and three dimensional analyses were compared by calculating the coefficient of correlation between corresponding dimensions in the two models. Each of the 32 sets of recorded data was treated in this way: the mean value of this coefficient for radial coordinates is 0.989 (S.D. = 0.01;  $n = 32$ ); and for circumferential coordinates, 0.987 (S.D. = 0.02;  $n = 32$ ). Both results confirm that remarkably little distortion of the overall retinotopy is caused by the usual assumption that the tectum is a flat, horizontal surface. (Inevitably the distortion which does derive from this affects especially the part of the tectum closest to the edge of the horizontal projection). It is therefore appropriate to consider the results of a flat tectal analysis alongside those of the more complex method; and in future studies it may be adequate to limit the mathematical manipulation of data to the two-dimensional model, if this is more convenient.

#### COMPARISON OF VISUAL FIELD AND TECTAL COORDINATES.

The method used to analyse polar coordinate visuotectal maps may be illustrated with reference to a map showing normal topography (figure 68a). Figure 68b shows a scatter diagram of the circumferential coordinates of optimum stimulus directions in the visual field ( $C_v$ )

plotted against the circumferential coordinates of tectal recording sites ( $C_t$ ). Figure 68c shows a similar diagram for radial coordinates ( $R_v$  against  $R_t$ ).

Simple regression of the abscissa on the ordinate in each scatter diagram produces a regression equation of the form " $y = a + b x$ " for the straight line of best fit (i.e. with the least sum of the squares of the deviations of points from this line) ( $y$  = abscissa,  $x$  = ordinate,  $a, b$  are constants). The equations for this example are:  $y = 1.74 + 1.09x$  (circumferential) and  $y = 8.56 + 0.99x$  (radial). This line is used to calculate  $r^2$  (expressed as a percentage), which indicates the proportion of the variation in the visual coordinate ( $C_v$  or  $R_v$ ) which is attributable to its correlation with the corresponding coordinate on the tectum ( $C_t$  or  $R_t$ ). From  $r^2$ , the correlation coefficient " $r$ " is obtained. Appendix D summarizes the calculations: for full explanation see Draper and Smith (1981).

In this example,  $r = 0.97$  (circumferential),  $r = 0.90$  (radial). The standard error of the correlation coefficient is calculated from:

$$S.E. = (1 - r^2) / n^{1/2}$$

where  $r$  = correlation coefficient,  $n$  = number of pairs of coordinates; and the " $t$ " statistic given by :

$$t = \frac{r (n - 2)^{1/2}}{(1 - r^2)}$$

is used to find the probability that this coefficient could occur by chance in a sample of  $n$  pairs from a population whose true coefficient is zero (Swinscow, 1980). (The " $t$ " table is entered at  $n - 2$  degrees of freedom.) In this example,  $P \ll 0.001$  for both dimensions. (All these calculations were performed using the "Minitab" statistics package of the Imperial College Computer Centre, University of London).

The practical justification for using a straight line regression equation rests on a subjective estimation of how well a straight line

fits each individual scatter diagram. The mathematical principle is of making a tentative assumption and subsequently assessing whether it was reasonable. This is aided by plotting the standard residuals of the points against the observed and against the predicted values of the visual field coordinates. (The standard residual is a measure of the difference between the observed and predicted values of the coordinates). If a straight line is a reasonable approximation the standard residuals appear to be normally distributed about zero at all values of the coordinate. This is the pattern of residual plots for almost all the regression equations calculated below: a single example is shown to illustrate the pattern (figure 68d). It is usual not to emphasize statistical techniques for the examination of residuals: it is held that "in practical situations a detailed examination of the corresponding residual plots is usually far more informative, and the plots will almost certainly reveal any violations of assumptions serious enough to require corrective action" (Draper and Smith, 1981, p.150). Curves in the original plot which might be overlooked are made more obvious by this plot of standard residuals: examples are noted below. Residual plots also serve to highlight individual observations set apart from the rest: such points are termed "outliers", and merit special attention.

## NOTES ON THE INTERPRETATION OF CALCULATED DATA

### A) INACCURACY DUE TO CURVATURE OF THE TECTUM

Figure 68e shows a graph of the modulus of the standard residual, plotted against the distance within the horizontal projection of the tectum from the estimated centre of this projection to the recording site. (The data are from a map with normal topography, but the principle applies to all maps.) It is clear that there is an increase in the modulus of the standard residual as the perimeter of the horizontal projection of the tectum is approached. This is to be expected from the experimental procedure: error in the horizontal coordinate while positioning the microelectrode (an error which is presumably normally distributed with the same standard deviation throughout the range of the micromanipulator) gives rise to an error in the positioning of the electrode tip within the curved synaptic lamina of the tectum, which is greater the further the recording site is from the horizontal part of the tectum.

An equivalent problem exists in the two-dimensional model of the tectum usually employed, because in making this projection from the curved tectal surface (appendix A) visual space represented on the peripheral part of this projection becomes relatively compressed. Both two- and three- dimensional maps are therefore likely to be less precise in this region. In theory one could compensate for this by weighting the correlation of radial coordinates to reduce the importance of recording sites near the vertical part of the tectum, and attention to this point is one way in which the present model could possibly be improved.

### B) REGRESSION EQUATION: RADIAL DIMENSION

The regression equation derived from each scatter diagram of visual field and tectal radial coordinates requires careful interpretation. In the straight line regression equation " $y = a + b x$ ", "a" is the

intersection of the regression line on the "y" axis. Taken literally, if "a" is positive it indicates that central visual field is unrepresented on the tectum; and if negative, this should mean that the optic axis is represented by a ring on the synaptic lamina enclosing a vacant area. Examples of this are discussed below, in relation to the experimental treatment of those fish. In normal fish one expects the regression equation to pass through the origin, and in fact "a" generally is close to zero (e.g.  $a = 8.56$  in the example above). Small positive or negative values may be explained by local deviations from the straight regression line. A regression line derived from mapping points elsewhere on the tectum does not provide a good basis for extrapolation to central tectum; and the only acceptable evidence that there is either a central scotoma or a patch of unoccupied tectum must be from the central recording sites themselves.

The other constant in the regression equation, "b", is the gradient of the line. If this is unusually high it indicates that that map is relatively compressed in the radial dimension. There is one interesting example of a partial map with a very low value of "b", indicating radial expansion. This is remarked upon below, in discussion of map A17D72.

#### C) TOPOGRAPHY: RADIAL DIMENSION

The definition of topography in this set of neural connections is that the optic axons from adjacent ganglion cells should synapse in adjacent areas of the tectum. To fulfil this definition, the areas need not be the same shape in the two laminae: an hypothetical lattice of squares on the retina could be transformed into a lattice of parallelograms on the tectum. Nor is it mandatory for the distribution of axons' terminals to take the same shape as the distribution of ganglion cell bodies: a hemispherical retina could transpose onto a spheroidal tectum (e.g. one the shape of a rugby ball). This would distort the



relative positions of the elements in the projection, but need not disrupt neighbour relations.

Since topography per se does not require uniformity in the set of retinotectal connections, it is obvious that perfect correlation of the radial coordinates is only a special case and that the visuotectal map can be topographic without conforming to this particular model. This point may be illustrated by taking a simple alternative model in which equal annuli on the retina project to unequal annuli on the tectum: the topography in the radial dimension might then be perfect yet not fit a straight line regression equation. Since there is no reason at all to assume that such annuli must be equal it is theoretically better to assess the precision of topography from the Rank correlation of the retinal and tectal coordinates, rather than the coefficient "r". The Rank correlation coefficient assesses the concordance between the sequence of tectal loci listed in order of ascending radial coordinates and the sequence of visual stimulus directions, similarly listed. No regression equation is produced. The actual value of each coordinate is irrelevant. (Kendall's method is used: see Swinscow (1980) if illustration is required.)

Comparison of the two coefficients derived from coordinate value correlation and coordinate Rank correlation provides a means of assessing how well the observed pattern of connections actually fits the former special case. In this example (figure 68c) the similarity of product moment (0.90) and Rank (0.91) coefficients indicates that the radial dimension of the visuotectal map is highly uniform.

This interesting question may be investigated further by inspecting the residual plots to discover whether points with positive residuals occur consistently on one side of the map, and those with negative, on the other. This pattern is observed in figure 61d: points with positive residuals lie in caudal tectum, those with negative lie rostrally. There

are two possible explanations for this. One is that the estimated position on the tectum of the projection of the papillary axis (A') is too far rostral. The other is that if A' is correctly placed, the map shows non-uniform topography: the representation of nasal visual field is relatively compressed, or that of temporal field is relatively expanded. Differentiation between these possibilities relies on being able to decide with confidence whether A' is correctly placed. In R168a (figure 61a) three recording sites (25, 26, 27) all had optimum stimulus directions close to the papillary axis. If reliance is placed on these one might place A' between site 26 and 27, and thus obtain the computed values of tectal polar coordinates shown in figures 61b and 61c, and the striking asymmetry of residuals already referred to (figure 61d). But judging from the mapping of surrounding points, one would expect (presuming topography) that A' should be midway between tectal sites 19 and 30, and midway between 20 and 29. If this revised estimate is used for A' and new polar coordinates calculated (figures 61e, 61f), the asymmetry of residuals disappears, and the correlation coefficient for the radial dimension increases to 0.941 from the value of 0.811 obtained using the first A'. This favours the former of the two explanations for the initial result. None of the present maps show this type of asymmetry of residuals accompanied by firm evidence from the mapping of central visual field about the position of A'. Therefore there is no relative expansion or compression of different sectors of the map.

This is most significant: it follows from the definition of topography that circular annuli on the retina need not be imaged on the tectum by annuli of a similar shape. This form of topography would be very difficult to quantify: if circles are distorted into ovals even the Rank correlation analysis is inappropriate. In such a case the only reliable index of orderliness would be a Rank correlation analysis across a single diameter of the tectum. This would be of very limited value

unless a large number of closely spaced recording sites along this line were used; and even then it would have to be repeated for many different diameters in order to sample the entire tectum.

Arguing from the result to support the assumptions on which it is based (cf. Draper and Smith, 1981) the high values of the correlation coefficients obtained do indicate that the special, uniform type of topography proposed in this model is, in fact, appropriate in almost every case.

#### D) REGRESSION EQUATION: CIRCUMFERENTIAL DIMENSION

The regression equation for the circumferential coordinates also has the form " $y = a + b x$ ". The value of "a" is affected by the arbitrary choice of the rostral direction as the reference axis from which the circumferential coordinate is measured. This choice is approximately correct: values of "a" for normal and regenerated projections span the range -10.5 to 68.6, mean = 23.5, S.D. = 19.8. Because the precise orientation of the visuotectal map is irrelevant to the topography it displays, no importance is attached to the value of "a" in these experiments.

Although this information about the variation in "a" is incidental to the present investigation, it could be used as an index of how precisely the orientation of the visuotectal map is controlled. This might help the investigation of the mechanism responsible. For values of "a" to be useful, however, it would be essential to measure the orientation of the eye (i.e. the angle between the ventral vestige in the sclera of the eye-cup fissure and the vertical) with greater precision than was the case in these experiments (the method having <sup>been</sup> devised after the maps were produced).

The other constant in the equation, "b", should be approximately equal to 1. Deviations from this imply angular expansion and compression. If "b" is greater or less than 1 there must be a compensatory opposite

deviation from 1 in optic projection on the unmapped ventral part of the tectum, unless a sector of tectum is uninnervated. As with the radial coordinate, the fact that uniformity is not required for topography makes it preferable to use a Rank correlation analysis to assess the precision of order. For each map the Rank coefficient is calculated using Kendall's method. In the example being used to illustrate these points (figure 68), the value is 0.98. This is very little different from the correlation coefficient "r" (0.97), showing that there is remarkable uniformity in the circumferential coordinate too: evidence that there is not merely topography, but a special form of topography, with very uniform representation of the visual field in both dimensions.

#### E) ERRORS IN CIRCUMFERENTIAL COORDINATES OF CENTRAL POINTS

The map shown in figure 61a has already been used as an example to illustrate the effect of mis-identifying the projection of the papillary axis (A'). It also serves to illustrate the susceptibility of the correlation coefficient in the circumferential dimension to local errors in the mapping of the central visual projection. Figure 61e shows the scatter diagram of visual field and tectal circumferential coordinates, the latter calculated using the revised position of A'. All the points fall close to a single straight line of proportionality except for 26 and 27. Tectal coordinates for these are about 180 degrees higher than expected; but what appears to be a gross error of topography may be attributed to a very modest error in the absolute position of the central projection, such that these points fall on the wrong side of A' (figure 61a).

The measurement of circumferential coordinates in the visual field is necessarily less accurate the lower the radial coordinate, which provides another reason for points such as these to deviate from the regression line indicated by more peripheral points (but not necessarily

by 180 degrees).

If points 26 and 27 are excluded from the computation of the correlation coefficient, either on the grounds that they are merely local errors of topography, or because they are not reliably mapped, the coefficient increases from 0.947 to 0.987.

Similar comments apply to point 49 in map R166a (figure 58a) and point 15 in map R166b (figure 59a); and in general the coefficient of correlation in this dimension must underestimate the precision of the topography present in the non-central part of the map. Individual maps are unequally affected by these problems because they vary in the number of elements mapped in the central part of the projection. It could be that suitable weighting of each observation might reduce this distortion of the calculated coefficients.

#### F) MAPPING IN AIR

These visuotectal mapping experiments were performed with the eye in air. Refraction of light entering the eye occurs at the air-cornea interface, such that optimum stimulus directions are further from the optic axis than they would be if mapping were performed with the eye under water. If the radius of curvature of the cornea is different near the limbus from that at its centre (Schmidt, Cicerone and Easter, 1978) one may expect this distortion to be different at different radii (a practical point reinforcing the theoretical one above in favour of studying rank correlation). Visuotectal topography per se is unchanged by this refraction.

## APPENDIX A

### EVIDENCE SUPPORTING THE ADOPTION OF A SPHERICAL MODEL OF THE TECTUM

Plates 3 and 4 show coronal and horizontal sections through goldfish tecta, and illustrate the curvature of the structure. In each section the tectal laminae appear to conform approximately to arcs of concentric circles. To show the overall curvature in three dimensions it would be necessary to reconstruct the tectum from serial sections. Histological sections can be distorted in the process of fixation and embedding, so a more accurate measurement of tectal curvature in vivo may be obtained by a modified electrophysiological mapping procedure.

Three fish were used, prepared as for visuotectal mapping. A microelectrode mounted on a micromanipulator was used to touch 20 to 30 points on the surface of the tectum. These positions were recorded in Cartesian coordinates in three axes: up, rostral and medial. The latter two coordinates were used to plot a horizontal projection of these surface loci, for each fish (figures 32, 34, 36). The microelectrode was also used as a probe to locate points around the edge of accessible tectum. The medial border of the tectum is a straight line. As with the tectal boundaries of the visuotectal maps (figures 50a to 78a) the arc of a circle has been fitted, as closely as possible (by subjective judgement) to the points (marked "x" in figures 32, 34, 36) indicating rostral, lateral and caudal extremities. The radius "r" of this arc was measured. The centre of the arc is used as an estimate of the horizontal projection of the centre of the tectum. Caudomedially, where the tectum abuts the hindbrain, the true edge of the tectum is further rostral than the drawn arc.

The distance within the horizontal projection of each mapped point from the centre of this arc was calculated and plotted as the "x" coordinate in each of three graphs (figures 33, 35 and 37). The "y" coordinate in these graphs is the measured vertical coordinate of each

mapped surface locus. Each locus is identified on the diagram of the tectum and on the graph by an alphanumeric code (e.g. "a 3") at the appropriate coordinate values. Thus, if the mapped points are conceived as lying on many different lines of "longitude" on the surface of a tectum whose dorsalmost point represents the "North Pole", each graph is an angular projection of these lines of longitude on to a single plane. This allows the overall curvature of the three dimensional tectum to be portrayed, rather than simply the curvature in a single coronal or parasagittal section.

It is immediately obvious that most of the points lie on or close to the arc of a circle whose radius is "r", the radius of the horizontal projection. The alphanumeric codes employed allow easy identification of the points furthest from this arc: in each case the only consistent deviation from the line is of the caudal-most points, indicating some flattening of the tectum close to its junction with the hindbrain. With this exception it is evident that points along all the lines of longitude describe a sphere of radius "r" (at least more accurately than they describe any other simple shape). Modelling an idealized tectum on this shape is therefore the best approximation.

Histological and electrophysiological studies have shown that the part of the tectum receiving the retinal input comprises concentric laminae (e.g. Schmidt, 1979; Meyer, 1980; Meek, 1981; Stuermer, 1984). The lamina of tectal recording sites used in the experiments reported here must therefore also conform closely to a sphere. The surface of the tectum rather than the lamina of recording sites was used to demonstrate this curvature because the vertical coordinate of the tectal surface may be more accurately determined.

## APPENDIX B

### COMPUTATION OF POLAR COORDINATES "r", "c" FOR A SPHERICAL TECTUM

In the following algebra:

O = Centre of tectal sphere

f axis = dorsal

g axis = rostral

h axis = medial

(See figure 38)

n = radius of tectal sphere (i.e. lamina of recording sites)

A' = projection of optic papillary axis on surface of tectum

G = Rostral extremity of tectal sphere

S' = a recording site, the projection of visual stimulus "S" on the tectum.

(See figure 39)

(  $f_a, g_a, h_a$  ) are Cartesian coordinates of A',  
in axes f, g, h.

(See figure 40)

Likewise,

(  $f_g, g_g, h_g$  ) are Cartesian coordinates of G,

(  $f_s, g_s, h_s$  ) are Cartesian coordinates of S'

The "f" coordinates are calculated from the measured "g, h" coordinates:

$$n^2 = f^2 + g^2 + h^2$$
$$\therefore f = ( n^2 - g^2 - h^2 )^{1/2}$$



A simple analogy clarifies the nature of the transformation of the three Cartesian coordinates. The tectum may be envisaged as part of a globe: A' is the north pole, on the radius OA'. S' is a point on the surface of the globe: its radial coordinate is 90° minus its latitude, i.e. the angle A'OS'. This is readily calculated. The circumferential coordinate of S' is its longitude, measured from an arbitrary meridian through G. Calculation of the circumferential coordinate of S' is achieved by first transforming the three orthogonal axes f, g, h into three orthogonal axes x, y, z, to obtain new Cartesian coordinates for S' ( $x_s, y_s, z_s$ ).

#### CALCULATION OF THE RADIAL COORDINATE OF "S' ":

Radial coordinate of S' = "r" in figures 40, 41.

$$(A'S')^2 = (OA')^2 + (OS')^2 - 2*(OA')*(OS')*\cos r$$

(Cosine law)

$$OA' = OS' = n$$

$$\therefore (A'S')^2 = 2n^2 * (1 - \cos r)$$

$$\therefore \cos r = 1 - [(A'S')^2] / 2n^2$$

$$(A'S')^2 = (f_a - f_s)^2 + (g_a - g_s)^2 + (h_a - h_s)^2$$

Hence "r" is calculated easily.

# CALCULATION OF THE CIRCUMFERENTIAL COORDINATE OF " S' ":

The circumferential coordinate of S' is defined as the angle "c" between the plane OA'S' and the plane OA'G (page 69). See figures 41,42.

This may conveniently be calculated by first re-expressing the position of S' in terms of a new set of three-dimensional Cartesian axes, x, y, z.

x axis = OA' produced.

y and z axes are perpendicular to x and to each other.

The plane defined by the x and y axes contains OA'G.

(see figure 42)

∴ c = angle between plane OA'S' and the y axis

(  $x_a, y_a, z_a$  ) = coordinates of A'

(  $x_g, y_g, z_g$  ) = coordinates of G

(  $x_s, y_s, z_s$  ) = coordinates of S'

If coordinates  $y_s$  and  $z_s$  are calculated, "c" may be easily obtained.

(See figure 43)

Calculation of  $y_s$  and  $z_s$  may be performed in the following stages:

The three points, O, A', S' whose coordinates are known in both sets of axes fix the three-dimensional relationship between f, g, h and x, y, z, for the tectum in question.

Equivalent coordinates:

POINTS	AXES			AXES		
	f	g	h	x	y	z
O	( 0	0	0 )	( 0	0	0 )
A'	( $f_a$	$g_a$	$h_a$ )	( n	0	0 )
G	( 0	n	0 )	( $x_g$	$y_g$	0 )

Let  $w$  = Angle between  $A'O$  and  $OG$  : (See figure 44)

$$(A'G)^2 = (OA')^2 + (OG)^2 - 2 * (OA') * (OG) * \cos w$$

(Cosine Law)

$$OA' = OG = n$$

$$\therefore \cos w = 1 - (A'G)^2 / 2n^2$$

$$\text{where } (A'G)^2 = (f_a - f_g)^2 + (g_a - g_g)^2 + (h_a - h_g)^2$$

$$= f_a^2 + (g_a - n)^2 + h_a^2$$

Hence calculate  $w$

(Calculation of  $x_g, y_g$  :

$$x_g = OP = n * \cos w ; \quad y_g = OQ = n * \sin w ;$$

but these values may be omitted:  $w$  is used.)

The next step is to find an algebraic expression giving

$y_s$  and  $z_s$  in terms of  $f_s, g_s$ , and  $h_s$ .

A vectorial method is proposed in which expressions are first described for unit vectors along  $x, y$ , and  $z$  axes as functions of  $f, g$ , and  $h$  coordinates.

(The mathematics have been taken from Bak and Lichtenberg, 1966)

Let  $\underline{i}$ ,  $\underline{j}$ ,  $\underline{k}$  be unit vectors in  $f$ ,  $g$ ,  $h$  axes respectively.

(See figure 45)

$\underline{OA'}$  is a vector in the  $x$  axis from 0 to the point with coordinates

$$(f_a, g_a, h_a)$$

(see figures 46, 47)

$$\underline{OA'} = (f_a \underline{i} + g_a \underline{j} + h_a \underline{k})$$

$$\text{Magnitude } |\underline{OA'}| = n = (f_a^2 + g_a^2 + h_a^2)^{1/2}$$

$\underline{OQ}$  is a vector in the  $y$  axis from 0 to the point with coordinates

$$(f_q, g_q, h_q).$$

(see figure 48)

$$\therefore \underline{OQ} = (f_q \underline{i} + g_q \underline{j} + h_q \underline{k})$$

$$\text{Magnitude of } \underline{OQ} = (f_q^2 + g_q^2 + h_q^2)^{1/2} = s$$

$$\underline{OQ} = \underline{OG} - \underline{OP}, \quad \underline{OP} = (\cos w) * \underline{OA'}$$

(see figure 44)

$$\therefore \underline{OQ} = (O\underline{i} + n\underline{j} + O\underline{k}) - (\cos w) * (f_a \underline{i}, g_a \underline{j}, h_a \underline{k})$$

$\therefore$  having calculated  $w$ , values of  $f_q, g_q, h_q$  and  $s$  are readily obtained.

OZ is a vector in the z axis, defined as the vector product

$$\underline{OA'} \times \underline{OQ}$$

(See figure 46)

The magnitude of OZ is  $|\underline{OA'}| * |\underline{OQ}|$ .

$$\begin{aligned} \underline{OZ} &= \underline{OA'} \times \underline{OQ} = (f_a \underline{i} + g_a \underline{j} + h_a \underline{k}) \times (f_q \underline{i} + g_q \underline{j} + h_q \underline{k}) \\ &= f_a * f_q (\underline{i} \times \underline{i}) + f_a * g_q (\underline{i} \times \underline{j}) + f_a * h_q (\underline{i} \times \underline{k}) \\ &\quad + g_a * f_q (\underline{j} \times \underline{i}) + g_a * g_q (\underline{j} \times \underline{j}) + g_a * h_q (\underline{j} \times \underline{k}) \\ &\quad + h_a * f_q (\underline{k} \times \underline{i}) + h_a * g_q (\underline{k} \times \underline{j}) + h_a * h_q (\underline{k} \times \underline{k}) \end{aligned}$$

This simplifies:  $\underline{i} \times \underline{i} = \underline{j} \times \underline{j} = \underline{k} \times \underline{k} = 0$ ,

and  $\underline{i} \times \underline{j} = \underline{k}$ ,  $\underline{j} \times \underline{k} = \underline{i}$ ,  $\underline{k} \times \underline{i} = \underline{j}$ ,

$\underline{j} \times \underline{i} = -\underline{k}$ ,  $\underline{k} \times \underline{j} = -\underline{i}$ ,  $\underline{i} \times \underline{k} = -\underline{j}$ ,

(by the definition of vector products),

$$\begin{aligned} \therefore \underline{OZ} &= \underline{OA'} \times \underline{OQ} = (g_a h_q - h_a g_q) \underline{i} \\ &\quad + (h_a f_q - f_a h_q) \underline{j} \\ &\quad + (f_a g_q - g_a f_q) \underline{k} \end{aligned}$$

If  $\underline{OZ} = (f_z \underline{i} + g_z \underline{j} + h_z \underline{k})$  (See figure 49),

the values of coordinates  $(f_z, g_z, h_z)$  may be calculated:

$$f_z = (g_a h_q - h_a g_q)$$

$$g_z = (h_a f_q - f_a h_q)$$

$$h_z = (f_a g_q - g_a f_q)$$

The magnitude of OZ =  $t = (f_z^2 + g_z^2 + h_z^2)^{1/2}$

From the three vectors  $\underline{OA'}$ ,  $\underline{OS}$  and  $\underline{OZ}$  it is simple to derive expressions for unit vectors  $\underline{i'}$ ,  $\underline{j'}$ ,  $\underline{k'}$  in the new axes, x, y, z respectively:

$$\text{Unit vector in x axis} = \underline{i'} = \underline{OA'}/n = \left( \frac{f_a}{n}, \frac{g_a}{n}, \frac{h_a}{n} \right)$$

$$\text{Unit vector in y axis} = \underline{j'} = \underline{OQ}/s = \left( \frac{f_q}{s}, \frac{g_q}{s}, \frac{h_q}{s} \right)$$

$$\text{Unit vector in z axis} = \underline{k'} = \underline{OZ}/t = \left( \frac{f_z}{t}, \frac{g_z}{t}, \frac{h_z}{t} \right)$$

Having obtained these unit vectors for each tectum, calculation of values of  $y_s$  and  $z_s$  for each mapped locus within that tectum is simple:

If a point S' had originally been described in terms of coordinates  $x_s$ ,  $y_s$ ,  $z_s$  (i.e. in the new axes), the coordinates of this point in the old axes would have been given by the vector transformation:

$$\begin{pmatrix} f_s \\ g_s \\ h_s \end{pmatrix} = \begin{pmatrix} \underline{i'}, \underline{j'}, \underline{k'} \end{pmatrix} \begin{pmatrix} x_s \\ y_s \\ z_s \end{pmatrix}$$

This is obviously the reverse of the transformation actually required, from old to new coordinates; but by vector substitution and matrix manipulations (or by algebra) this solves for:

$$\begin{aligned} x_s &= f_a f_s / n + g_a g_s / n + h_a h_s / n \\ y_s &= f_q f_s / s + g_q g_s / s + h_q h_s / s \\ z_s &= f_z f_s / t + g_z g_s / t + h_z h_s / t \end{aligned}$$

Of these, only  $y_s$  and  $z_s$  are calculated: see figure 43.

Calculation of "c":

$$\tan v = z_s / y_s$$

v has values  $-90^\circ$  to  $+90^\circ$

"c" has values 0 to 360 : c is calculated from v

by considering which quadrant v lies in:

- i) If  $y > 0$  and  $z > 0$  ,  $c = v$
- ii) If  $y < 0$  and  $z > 0$  , v is negative and c lies in the quadrant  $90$  to  $180^\circ$ , so:  $c = v + 180^\circ$
- iii) If  $y < 0$  and  $z < 0$  , v is positive and c lies in the quadrant  $180$  to  $270^\circ$ , so:  $c = v + 180^\circ$
- iv) If  $y > 0$  and  $z < 0$  , v is negative and c lies in the quadrant  $270$  to  $360^\circ$ , so:  $c = v + 360^\circ$

Note that a visual stimulus S with circumferential coordinate  $c = 5^\circ$  may project to a tectal locus S' with  $c = 355^\circ$  (for example) in a perfectly topographic map, the apparent difference being due to arbitrary rotation of one map relative to the other by  $10^\circ$ . This would distort the true value of the correlation coefficient for the circumferential coordinates, and must be avoided by allowing c to take values " $c + 360$ " or " $c - 360$ " instead. This requires the circular argument that values of c are chosen because the map is topographic in the circumferential dimension; but this is acceptable because the general orderliness of the projection is obvious on inspection, and the values of c are being used only to measure the precision of the pattern. It is assumed that the map is sufficiently orderly for any pair of circumferential coordinates to be within  $180^\circ$  of each other. (This would be a weakness in this method of analysis if applied to a map in which there was reversal of polarity).

## APPENDIX C

### COMPUTATION OF POLAR COORDINATES "r", "c" FOR A HORIZONTAL PLANAR MODEL TECTUM.

The radial coordinate "r" is defined as the distance within the plane from the horizontal projection of the optic axis (A') to the horizontal projection of the recording site (S').

The circumferential coordinate is defined as the angle between the line A'S' and the reference line arbitrarily chosen as the parasagittal rostral direction from A'.

"r" and "c" are readily calculated from g, h, by simple algebra and trigonometry.

### COMPUTATION OF POLAR COORDINATES FOR A FLAT HORIZONTAL TECTAL MODEL FOR A "PERFECT" MAP (see text page 71).

For each mapped tectum the measured coordinates of the recording sites are used to calculate polar coordinates for both three and two dimensional model tecta. A "perfect" (fictitious) visuotectal map is defined as one in which there is perfect product-moment correlation between the polar coordinates of the visual stimuli and the polar coordinates of the recording sites in a three dimensional model tectum. There might, for example, be identical coordinates for corresponding visual and tectal positions.

Using three dimensional tectal coordinates as if they were optic stimulus directions, they may be correlated with the calculated polar coordinates for the planar model tectum. Any reduction from the value 1 represents error due to neglect of the curvature of the tectum.



## APPENDIX D

### CALCULATION OF PRODUCT MOMENT CORRELATION COEFFICIENTS

It is necessary to find an index of how accurately the points on each scatter diagram fit the regression line  $Y = a + bx$

Let:  $Y_i$  be the observed value of a coordinate,  
 $\hat{Y}_i$  be the predicted value, and  
 $\bar{Y}$  be the mean of the  $Y_i$  values.

Starting from the identity

$$Y_i - \hat{Y}_i = Y_i - \bar{Y} - \hat{Y}_i + \bar{Y}$$

it can be shown that

$$(Y_i - \bar{Y})^2 = (Y_i - \hat{Y}_i)^2 + (\hat{Y}_i - \bar{Y})^2, \text{ where}$$

$$(Y_i - \bar{Y})^2 = \text{sum of the squares of deviations from the mean;}$$

$$(Y_i - \hat{Y}_i)^2 = \text{sum of the squares of deviations of observed from predicted values;}$$

$$(\hat{Y}_i - \bar{Y})^2 = \text{sum of the squares of deviations of the predicted values from the mean}$$

(cf. Draper and Smith, 1981)

Writing these terms in their customary abbreviations,

Sum of squares		
	=	+
about the mean		about regression      due to regression.

Consideration of how much of the sum of squares about the mean is attributable to the sum of squares due to regression and how much to the sum of squares about the regression provides a means of assessing how useful the regression line is as a predictor - or to put it another way, how well the two coordinates correlate. If the points fit the line closely the sum of squares <sup>0</sup>about the regression is small, so the ratio

$$r^2 = \frac{\text{Sum of squares due to regression}}{\text{Sum of squares about the mean}}$$

will be nearly unity.

The square root of this ratio,  $r$ , (which takes the sign of "b", positive in all these maps) is the correlation coefficient in the model regression situation, where it is assumed that the values of  $X$  are not subject to random error, and  $R_v$ ,  $C_v$  are random about mean values specified by the model  $Y = a + bX$ .

# APPENDIX E

## COMPUTER PROGRAM ( BASIC ) FOR CALCULATION OF POLAR COORDINATES FOR THREE DIMENSIONAL TECTUM

```

0100 PROGRAM V (RAW, OUT8, INPUT, OUTPUT
0110 +TAPE7=RAW, TAPE8=OUT8,
0120 +TAPE5=INPUT, TAPE6=OUTPUT)
0130 COMMON /A/A (99), B (99), G (99), H (99)
0140 COMMON /B/F (99), Y (99), Z (99), V1 (99), V2 (99)
0150 COMMON /C/C (99), RS (99)
0160 COMMON /D/TX (99), TY (99), RX (99), RY (99), CP (99), RP (99)
0170 COMMON /E/GG (99), HH (99)
0180 INTEGER S
0190 REAL MCW, M, L
0200 PI = 3.141592654
0210 2 CONTINUE
0220 READ (7, 110) DATE
0230 110      FORMAT (A10)
0240 READ (7, *) NO, NX
0250      NT = NO + NX
0260 READ (7, *) R, GA, HA
0262 C
0264 C      NEW      AXES
0266 C
0270 FA = SQRT (R * R - GA * GA - HA *HA)
0280 AGAG = FA * FA + (GA - R) * * 2 + HA * HA
0290 MCW = AGAG/2/R/R-1
0300 FQ = MCW * FA
0310 GQ = MCW * GA + R
0320 HQ = MCW * HA

```

```

0330 M = SQRT (FQ * FQ + GQ * GQ + HQ * HQ)
0340 FZ = GA * HQ - HA * GQ
0350 GZ = HA * FQ - FA * HQ
0360 HZ = FA * GQ - GA * FQ
0370 L = SQRT (FZ * FZ + GZ * GZ + HZ * HZ)
0380 C
0390 DO 10 S = 1, NT
0400 C
0410 C
0420 C      CIRCUMFERENTIAL CO-ORDINATE
0430 C
0440 READ (7, *) A(S), B(S), G(S), H(S)
0450 F(S) = SQRT (R * R - G(S) * G(S) - H(S) * H(S))
0460 Y(S) = (FQ * F(S) + GQ * G(S) + HQ * H(S)) / M
0470 Z(S) = (FZ * F(S) + GZ * G(S) + HZ * H(S)) / L
0480      IF (Y(S) . EQ . 0) Y(S) = Y(S) + 0.0000001
0490 V1(S) = ATAN (Z(S) / Y(S))
0500 V2(S) = V1(S)
0510 IF (Y(S) . LT . 0) V2(S) = V2(S) + PI
0520 IF V2(S) . LT . 0) V2(S) = V2(S) + PI + PI
0530 C(S) = V2(S) * 180 / PI
0540 IF (A(S) - C(S) . GT . 180) C(S) = C(S) + 360
0550 IF (C(S) - A(S) . GT . 180) C(S) = C(S) - 360
0560 C
0570 C      RADIAL CO-ORDINATE
0580 C
0590 ASAS = (FA - F(S)) * * 2 + (HA - H(S)) * * 2
0600 RS(S) = ACOS (1 - ASAS / 2 / R / R) * 180 / PI
0610 C

```

```

0620 C          CARTESIAN CO-ORDINATES
0630 C
0640 TX(S) = RS(S) * COS (C(S) * PI / 180)
0650 TY(S) = RS(S) * SIN (C(S) * PI / 180)
0660 RX(S) = B(S) * COS (A(S) * PI / 180)
0670 RY(S) = B(S) * SIN (A(S) * PI / 180)
0680 C
0690 C          SIMPLE PLANAR METHOD
0700 C
0710 GG(S) = G(S) - GA
0720 HH(S) = H(S) - HA
0730 IF (GG(S) . EQ . 0) GG(S) = GG(S) + 0.000001
0740 RP(S) = SQRT (GG(S) * * 2 + HH(S) * * 2)
0750 CP(S) = ATAN (HH(S) / GG(S))
0760 IF (GG(S) . LT . 0) CP(S) = CP(S) + PI
0770 IF (CP(S) . LT . 0) CP(S) = CP(S) + PI + PI
0780 CP(S) = CP(S) * 180 / PI
0782 IF (CP(S) - A(S) . GT . 150) CP(S) = CP(S) - 360
0784 IF (A(S) - CP(S) . GT . 150) CP(S) = CP(S) + 360
0790 C
0800 C
0810 10 CONTINUE
0820 C
0930 C
0940 DO 50 S = 1, NT
0950 C
0960 WRITE (8, 108) A(S), B(S), C(S), RS(S), CP(S), RP(S)
0970 +, G(S), H(S), TX(S), RX(S), RY(S), S
1000 108 FORMAT (F5.1, F6.1, F7.1, 2F6.1, 3F7.1, 2F6.1, 2F7.1, I3)
1020 C

```

1030 50 CONTINUE

1040 C

1200 END

## CHAPTER SIX

### RESULTS AND DISCUSSION:

#### VISUOTECTAL MAPS OF NORMAL AND REGENERATED PROJECTIONS

Visuotectal maps are presented in figures 50 to 63. Figures numbered with the suffix "a" show conventional diagrams of the visual field (upper) and of the horizontal projection of the tectum (lower) (see pages 65 to 67). Each is followed by the corresponding scatter diagrams for circumferential (suffix "b") and radial (suffix "c") dimensions. Other graphs are included ("d/e/f") as necessary.

Results obtained from six normal fish are denoted by the alphanumeric code "N1" to "N6". Nine fish were mapped after regeneration following a single, complete orthogonal cut through the optic nerve in mid-orbit. These results are titled with a code "R", followed by a number denoting the duration of regeneration in days, and a suffix "a", "b" or "c" to distinguish individuals mapped after equal periods of regeneration (e.g. "R 168 b").

The fish are numbered as follows:

N	1	2	3	4	5	6						
R	58	70	166a	166b	167	168a	168b	168c				

#### 1) NORMAL FISH

The topography of each visuotectal map is assessed by eye as well as mathematically. None of the normal (non-regenerated) maps (figures 50a to 55a) contain elements that are grossly misplaced, and all appear to be topographic. This may be demonstrated conveniently by the orderly sequence of stimulus directions corresponding to adjacent tectal loci, as illustrated by lines 14 to 21 and 37 to 43 in figure 52a. Scatter

diagrams for radial and circumferential dimensions follow each of the visuotectal maps. The corresponding regression equations are presented in table 1. Calculated values of the product moment correlation coefficient ( $r$ ),  $r^2$  and the Rank correlation coefficient are in table 2.

TABLE 1      REGRESSION EQUATIONS:   NORMAL FISH

FISH		CIRCUMFERENTIAL	RADIAL
	figure	figure b	figure c
N1	50	$Y = 2.06 + 0.88X$	$Y = 15.4 + 1.33X$
N2	51	$Y = 42.2 + 0.95X$	$Y = -0.75 + 1.31X$
N3	52	$Y = -10.5 + 1.06X$	$Y = 8.13 + 1.22X$
N4	53	$Y = -2.90 + 1.03X$	$Y = 0.19 + 1.54X$
N5	54	$Y = 20.2 + 0.98X$	$Y = 6.89 + 1.24X$
N6	55	$Y = 35.0 + 0.73X$	$Y = 9.26 + 1.08X$



TABLE 2      NORMAL FISH

FISH		CIRCUMFERENTIAL DIMENSION				
		$r^2$	r	Rank	P	S.E.
	figure					
N1	50b	83.1%	0.91	0.96	<< 0.001	0.003
N2	51b	95.3%	0.98	0.91	<< 0.001	0.007
N3	52b	97.3%	0.99	0.99	<< 0.001	0.004
N4	53b	98.1%	0.99	0.98	<< 0.001	0.004
N5	54b	96.8%	0.98	0.99	<< 0.001	0.005
N6	55b	88.2%	0.94	0.92	<< 0.001	0.026

		RADIAL DIMENSION				
		$r^2$	r	Rank	P	S.E.
N1	50c	60.8%	0.78	0.81	<< 0.001	0.066
N2	51c	85.4%	0.92	0.93	<< 0.001	0.023
N3	52c	74.8%	0.86	0.86	<< 0.001	0.036
N4	53c	75.7%	0.89	0.83	<< 0.001	0.051
N5	54c	78.9%	0.86	0.88	<< 0.001	0.033
N6	55c	82.9%	0.91	0.89	<< 0.001	0.038

P = Probability of r occurring by chance in a similar-sized sample from a population whose true coefficient is zero;

S.E. = Standard error of r: see page 72.

## CIRCUMFERENTIAL DIMENSION

The values of  $P$  show that in every normal map the regression of visual field coordinates on tectal coordinates is highly significant. The values of  $r^2$  show that in four of the normal fish over 95% of the variation in the circumferential coordinate in visual space ( $C_v$ ) is attributable to its correlation with the circumferential coordinate on the tectum ( $C_t$ ). In N6 the value is 88.2%, but this result may be less reliable than the others: no part of the ventral half of the visual field is mapped in this fish, so it is very difficult to estimate the tectal locus (A') imaging the optic (papillary) axis. Error in this can clearly distort the regression. In this fish the gradient of the regression line is abnormally low (0.73, compared with a range of 0.88 to 1.06 in the other normal fish), which implies abnormal expansion of the tectal representation of the mapped part of the visual field (and consequently compression of the unmapped part). Differential expansion and compression of sectors of the visual field has not been reported and is not evident in any of the other normal fish. If it were a feature of visuotectal maps one would also expect that the rank correlation coefficient would be higher than the product-moment coefficient: and this is not observed. A more likely explanation is that the image of the optic axis (A') is too far medial in this instance. Because of this reservation it seems advisable to disregard this value of  $r^2 = 88.2\%$ , and accept as normal the range indicated by N2 to N5.

In N1 too, only half the visual field was mapped: no circumferential coordinates were measured in the range  $200^\circ - 350^\circ$ . In the scatter diagram (50b) points with coordinates above  $350^\circ$  are widely separated from the remainder. This exaggerates the correlation between visual field and tectal coordinates. If  $360^\circ$  are subtracted from each circumferential coordinate for points 4, 20, 25, 30 the scatter diagram is altered to figure 50e, and the correlation coefficient

diminishes from 0.99 to 0.91. The reason why this is lower than for N2 to N5 may be the same as proposed for N6: both emphasize the need to map all round the optic axis before attempting to quantify the precision of topography by this method.

The similarity of "r" and rank correlation coefficients indicates that equal sectors of visual space are represented uniformly at least on that part of the tectum accessible for recording. This is one indication that a straight line regression is appropriate. The fact that the values of "b" are close to 1.00 (range 0.95 to 1.06, excluding N1 and N6) is another indication of this, for it suggests that the map on the accessible part of the tectum is not greatly expanded or compressed relative to the projection on to ventral (inaccessible) tectum. This uniformity qualifies the description "topographic", which by itself does not require this feature.

Values of "a" range from -10.5 to +42.2. The precise orientation of the eye was not measured accurately, so it cannot be proven that variation of  $\pm 26.4^\circ$  from the mean orientation did not occur. This is to be regretted because the variation in "a" would provide information about the accuracy with which the polarity of the retinotectal projection is controlled.

#### RADIAL DIMENSION

As in the circumferential dimension the regression of the visual field coordinates on the tectal coordinates is statistically highly significant ( $P \ll 0.001$ ).  $r^2$  values range from 60.8% to 85.4%. Rank correlation coefficients are very similar to product moment coefficients. This indicates that equal increments in  $R_v$  throughout its range are represented on the tectum by equal increments in  $R_t$ . Examination of the residual plots (not reproduced) confirms this, with the sole exception of

N1, in which the graph of standard residuals plotted against the visual field coordinates displays a modest convexity upwards (figure 50d). It appears that peripheral visual field has a slightly more expanded representation on the tectum than central field. This is also the fish in which the rank correlation coefficient most exceeds the product moment coefficient, but this difference is still very modest: 0.03. Error in the estimation of A' could cause this; but it may be that a moderate degree of inhomogeneity in the radial coordinate must be accepted as normal. In the other five fish there is no sign that a straight line regression is inappropriate.

The absence of relative compression and expansion of different sectors of the map has been noted above. However, it does appear that when the projection from half a retina expands to occupy the whole tectum this property of the normal map is not retained: the maps of Schmidt, Cicerone and Easter (1978) show that the "magnification factor" (a measure of the visual angle corresponding to unit length in the horizontal projection of the tectum) is non-uniform in such cases in a way that cannot be attributed to the curvature of the tectum; and indicates expansion of the projection to caudal tectum. For the normal fish described here, it may be concluded that cones drawn about the optic axis in visual space are represented by circles drawn about A' on the tectum, rather than by ovals or irregular lines. Were it otherwise straight line regression in the radial dimension would not be an appropriate model of topography.

Tectal loci very close to the perimeter of the horizontal projection of the tectum occasionally lie far from the regression line: e.g. point 23 in N4 (figure 53c), point 16 in N6 (figure 55c). This was predicted above on the grounds that the curvature of the tectum exaggerates any error in the positioning of the recording electrode. However, judging from the maps and scatter diagrams for N4 and N6,

relatively few points are much affected, and there appears to be no need to weight the radial coordinates of the recording sites with a function of their horizontal distance from the centre when deriving a correlation coefficient (page 74).

Values of "a" in the regression equations for the radial coordinates are in the range -0.75 to +15.4: close to zero, as predicted and discussed above (page 74).

The precise values of "b" are irrelevant to topography. The figure of  $b_1 = 1.06$  in N6 may be an underestimate arising from the presumed error in A'. The other fish have "b" the range 1.22 to 1.54.

#### COMPARISON OF RADIAL AND CIRCUMFERENTIAL DIMENSIONS

Values of  $r^2$  are noticeably lower for the radial dimension than for the circumferential. The observed difference between the values of  $r^2$  is not likely to have occurred by chance (a paired "t" test indicates a 99% confidence level). One must conclude that in the normal visuotectal map topography is significantly more precise in the circumferential dimension than in the radial.

In seeking an explanation for this, it is necessary to investigate whether there are any reasons to disqualify any low values of  $r^2$  for radial coordinates as invalid. If A' is too far medial in N6 (as suggested above) this could produce a lower value of  $r^2$  for the radial dimension than is appropriate.

But since the value of  $r^2$  for this fish is the second highest, it certainly is not possible to attribute the generally lower values of

$r^2$  in the radial dimension to this.

Another possible reason for this difference between circumferential and radial dimensions could be that the model of strict uniformity of topography is less accurate for the latter. But in fact radial topography shows no local distortion from the model: neither annular expansion/compression, nor expansion of certain sectors relative to others, in N 2 - 6; and there is only minimal expansion of the periphery in N1.

The conclusion that circumferential topography is more precise than radial relates strictly to the map and not necessarily to the anatomy of the retinotectal connections: it cannot be proven from the results presented here that the measurement of radial coordinates on the tectum or in visual space is no less accurate than the measurement of circumferential coordinates. Prediction of the errors in each dimension is not possible: in both visual field and tectum the error in the circumferential coordinate depends on the radial coordinate; and in the tectum this in turn depends on the positions of A' and the recording site (see pages 77, 74).

The only solution to this problem would be to re-map one tectum many times in a short period, re-using the same Cartesian coordinates for the tectal recording sites, and hence obtain direct evidence about the relative accuracy of radial and circumferential measurements. One could then examine the difference in accuracy of the two dimensions of these maps and decide how much of the difference is attributable to the mapping procedure and how much to the anatomy of the retinotectal projection. This would be a difficult experiment to perform, however: the whole tectum would have to be mapped each time to obtain representative data, so the fish would have to be kept uniformly anaesthetized and immobile for several times the duration of a standard mapping experiment (3 - 4 hours).

Indirect evidence relevant to this debate is that correlation between the accuracy of topography in radial and circumferential dimensions for N2 to N6 is negative: the correlation coefficient between  $r^2$  values is -0.84 (product moment) or -0.80 (Rank) (these values are not statistically significant at the 5% confidence level). Supposing that the anatomy of the retinotectal projection is topographic with equal precision in radial and circumferential dimensions, one might expect that any feature of the process of visuotectal mapping which selectively diminishes only (or predominantly) <sup>apparent</sup> radial orderliness would have the consequence that the accuracy of radial retinotopy would still be directly related to the accuracy of circumferential order. This, evidently, is not the case. One must be careful not to overload with interpretation this lack of direct correlation, because only five fish are being compared. But this curious feature points towards another interesting conclusion: if the precision of topography in one dimension is unrelated to the precision of topography in the other, one possible explanation is that the two dimensions of topography are not manifestations of a single organizing process. This would not necessarily indicate that the mechanism generating radial topography is different from that generating circumferential topography; but this suggestion is obviously relevant to the question whether radial topography might depend on the sequence of arrival of axons.

## 2) VISUOTECTAL MAPS OBTAINED AFTER UNINTERRUPTED REGENERATION

Visual assessment of the maps obtained subsequent to a single orthogonal cut through the whole optic nerve (figures 56a to 63a) indicates that the overall patterns are essentially topographic. However, in a few fish it is possible to identify a few points not conforming to the general pattern: R58 (points 6, 10, 11), R70 (4, 13, 16), R166A (2,3), R166B (24), R167 (3), R168A (25), R168C (20). These are identified by appearing to fall on the wrong side of one or more of their neighbours. By simply looking at the maps it is difficult to decide whether it is these points or their neighbours <sup>which</sup> are misplaced, whether the error is radial or circumferential, and what the extent of the abnormality is. As with the normal maps, assessment is greatly facilitated by the scatter diagrams of each coordinate (figures 56b,c to 63b,c), interpreted with help from the residual plots. The regression equations for these maps are shown in table 3, and the corresponding values of  $r^2$  and the correlation coefficients are shown in table 4.



TABLE 3: REGRESSION EQUATIONS OF REGENERATED VISUOTECTAL MAPS

FISH		CIRCUMFERENTIAL	RADIAL
	figure	figure b	figure c
R58	56	$Y = 68.6 + 0.62X$	$Y = -2.69 + 0.99X$
R70	57	$Y = 9.70 + 1.09X$	$Y = 18.4 + 1.22X$
R166a	58	$Y = 31.2 + 0.96X$	$Y = -1.18 + 1.20X$
R166b	59	$Y = 14.4 + 0.99X$	$Y = 0.18 + 1.09X$
R167	60	$Y = 19.8 + 0.82X$	$Y = 18.4 + 1.17X$
R168a	61	$Y = 29.1 + 0.96X$ (figure e)	$Y = -5.37 + 1.45X$ (figure f)
R168b	62	$Y = 32.0 + 0.95X$	$Y = 9.73 + 1.31X$
R168c	63	$Y = 37.5 + 0.98X$	$Y = 7.11 + 1.14X$

TABLE 4:

## REGENERATED VISUOTECTAL MAPS

FISH		CIRCUMFERENTIAL DIMENSION				
	figure	$r^2$	r	Rank	P	S.E.
R58	56b	70.0%	0.84	0.87	<< 0.001	0.067
R70	57b	94.5%	0.97	0.97	<< 0.001	0.011
R166a	58b	96.8%	0.98	0.96	<< 0.001	0.004
R166b	59b	95.2%	0.98	0.96	<< 0.001	0.010
R167	60b	96.4%	0.98	0.99	<< 0.001	0.007
R168a	61e	89.7%	0.95	0.75	<< 0.001	0.018
R168b	62b	91.0%	0.95	0.99	<< 0.001	0.021
R168c	63b	96.6%	0.98	0.99	<< 0.001	0.006

## RADIAL DIMENSION

		$r^2$	r	Rank	P	S.E.
R58	56c	74.1%	0.86	0.85	<< 0.001	0.056
R70	57c	47.7%	0.69	0.67	<< 0.001	0.105
R166a	58c	94.1%	0.97	0.97	<< 0.001	0.008
R166b	59c	88.4%	0.94	0.94	<< 0.001	0.023
R167	60c	80.0%	0.89	0.86	<< 0.001	0.038
R168a	61f	88.6%	0.94	0.96	<< 0.001	0.020
R168b	62c	81.4%	0.90	0.94	<< 0.001	0.043
R168c	63c	81.0%	0.90	0.91	<< 0.001	0.034

## CIRCUMFERENTIAL COORDINATES

In every case the regression of visual field on tectal coordinates is highly significant ( $P \ll 0.001$ ). Values of  $r^2$  for these fish are in the range 89.7% to 96.8%, with the sole exception of R58 ( $r^2 = 70.0\%$ ). These may be compared with the range in the normal fish: 95.3% to 98.1% (excluding N1 and N6). This quantitation confirms that regeneration restores almost completely the precision of circumferential topography in visuotectal maps (cf. Gaze, 1959).

R58 appears to be a special case in two respects. The scatter diagram for this fish (figure 56b) shows that only two observations (6 and 11) deviate from the pattern shown by the other points. There is no obvious reason for their abnormality: they do not relate to central visual field, which is mapped less accurately (page 79). This fish was also unusual in having larger multiunit receptive field sizes (measured in the radial dimension) than other fish. For tectal loci 6, 9, 11, 14, 20, a visual stimulus up to  $30^\circ$  from the recorded optimum direction could evoke a detectable response; for point 10 at the caudal pole of the tectum the figure is  $50^\circ$ . (Normally the radius of the receptive field is not above  $10^\circ$ ). These receptive fields appeared to be enlarged radially more than circumferentially. This abnormality does not necessarily denote a loss of topography but it suggests that the map in R58 is not representative of a mature regenerated retinotectal projection.

As in the normal fish, rank correlation coefficients are not substantially different from the product-moment coefficients. This carries the same implication of uniformity of topography as in the normal fish described above.

In the regression equations for the circumferential dimension of the regenerated visuotectal maps (table 3), values of "a" range from 9.70 to 42.1 (excluding R58), implying that the orientation of these

regenerated maps is comparable to that of the normal maps ( $10.5 \leq a \leq 42.2$ ).

Values of "b" for regenerated maps are in the range 0.82 to 1.09 (excluding R58), similar to the range for normal maps ( $0.95 \leq b \leq 1.06$ , excluding N1 and N6). As in normal maps the fact that these values are close to 1.00 indicates uniformity in the tectal representation of visual space.

#### RADIAL DIMENSIONS

All the regressions are highly significant ( $P \ll 0.001$ ).  $r^2$  ranges from 47.7% to 94.1%. The two highest of these, R166A (figure 58c) and R166B (figure 59c), exceed the highest values obtained from normal fish, and two others, R168B (figure 62c) and R168C (figure 63c), produce values of  $r^2$  above the mean obtained in normal fish. These results establish the high precision that can be achieved in this coordinate after regeneration. As in the normal maps,  $r^2$  for radial coordinates is not as high as for circumferential coordinates (with the exception of R58 considered above).

It is a fundamental principle in this new method of assessing the accuracy of topography that each of the scatter diagrams must be carefully scrutinized before interpreting  $r^2$ , for this serves only as an index of overall topography and conceals local flaws in the maps. The strength of the method lies in the portrayal of topography in these diagrams; and the weakness is the potential for over-interpretation of the values of "r". The following examples illustrate points where care must be exercised.

#### R168B

In R168B (figure 62c;  $r^2 = 81.4\%$ ) the observation numbered 1 is the sole point lying off a distinct line defined by the others. With this exception, which must lower the value of  $r^2$ , the regression of radial coordinates is remarkably linear. There is no obvious reason for 1 to be different, but tectum rostral and caudal to this recording site was shown to be devoid of visually evoked responses, so there may be a local abnormality of tectum.

#### R168C

R168c has a similar value of  $r^2$  (81.0%), but no single outlying point can be identified (figure 63c). Instead, the general topography in the radial dimension appears less accurate. Scrutiny of the residuals (figure 63d) shows that points 21, 22, 23, have positive residuals and points 24, 25, 26, and 27 have negative. This suggests that A' is too far rostral; but for the adjacent line of points (14 to 20) the caudal loci have large positive residuals, implying that A' is too far caudal. This conflict of internal evidence and the lack of consistent indication for revising the position of A' is very useful, allowing the conclusion that the correlation between radial coordinates would not have been greatly improved by the choice of a different rostrocaudal coordinate for A'. A similar study of residuals confirms the choice of the mediolateral coordinate of A' too.

#### R168A

This map has already been considered in relation to estimation of the position of A' (page 77).

#### R167

The map from fish R167 gives a value of  $r^2 = 80.0\%$  for the radial dimension, similar to that from R168B and R168C. The pattern of the scatter diagram is different, however: a markedly linear distribution of

points from the central part of the map alters at high coordinate values, where a number of points are scattered to the right of the straight line projected from lower values (figure 60c). A graph of standard residuals plotted against the radial coordinates in the visual field (figure 60d) displays a marked convexity upwards. This might imply that a curved regression line is more appropriate than a straight line (cf. N1); but the fact that the rank correlation coefficient (0.86) is lower than the product-moment coefficient (0.89) argues against this.

One subjective interpretation of the scatter diagram is to conclude that points 2,3,10,11, and 18 lie too far to the right, but that with these exceptions a linear regression line is entirely appropriate. This idea might gain some support if a reason for the abnormal mapping of these points could be found. Of these points, those furthest from the imagined line (3, 10, 11, 18) correspond to rostral and caudal extremities on the tectum, so one possible explanation for the error in the mapping of these points (but not of 2) is that the estimated radius of the tectum is too small. Because the plotted points along the perimeter of the horizontal projection of the tectum do not conform exactly to the arc of a circle, there is an element of subjectivity in the estimation of the radius of curvature of the tectum. The range over which this radius may be varied is limited by the mapped perimeter points, so any error is necessarily small. Moreover, the radial correlation coefficient is relatively insensitive to alterations in the radius. Two estimates of the tectal radius were made for this fish. An increase of 8.13% from the first estimate of the radius to the second (which is the value used in calculating the radial coordinates plotted in figure 60c) produced an increase in  $r^2$  of only 1.3%, and in  $r$  of only 0.8%. Enlarging the radius still further would take it beyond the limits imposed by the measured perimeter points.

Additional evidence that an incorrect radius is not responsible for

the abnormal position of points 3, 10, 11, and 18 is apparent in an additional scatter diagram (figure 60e). This shows measured radial coordinates in the visual field plotted against corresponding coordinates on the tectum, here calculated on the (incorrect) assumption that the tectum is flat. Despite the distortion inherent in this model points 2, 3, 10, 11 and 18 still appear to be to the right of a line on which the other points lie. The radius of the tectum is irrelevant to radial coordinates on a flat model tectum, so an incorrectly estimated radius cannot be the cause of the observed pattern. For these reasons the proposed explanation for the abnormality of this map must be rejected.

Error in only these five points is not the only possible conclusion from the scatter diagram in figure 60c. Subjective judgement is required in deciding where the linear part of the distribution stops. Instead of supposing that only 2, 3, 10, 11 and 18 are off the line, one could conclude that only visual field within  $65^\circ$  of the optic axis is topographically represented on the tectum; or that there is no topography more than  $50^\circ$  from A'.

No comparable mixture of order and disorder in different parts of the radial dimension in a map has previously been described. It might be more obvious what significance should be attached to this result if the conditions in which it occurs can be defined by repetition. It seems likely to arise from abnormal neural connections rather than from abnormal optics in the eye (such as peripheral corneal opacity) because the multiunit receptive field sizes of these misplaced projections are entirely normal.

It is important to note that loss of precision in the radial coordinates of these observations is not accompanied by loss of precision in the circumferential coordinates (figure 60b). This is interesting circumstantial evidence that radial and circumferential dimensions of the map are controlled independently.

R70

Map R70 (figure 57a) is most unusual. The caudal half of the tectum is almost entirely devoid of visual innervation, as is the lateral extremity. The temporal half field is fully represented on the tectum, however. The map appears to be displaced over the rostral pole of the tectum rather than simply compressed, since of the nasal half field only the central portion is represented on the dorsal aspect of the tectum, and that is at the rostral pole.

R70 presents the most striking contrast between the accuracy of the circumferential topography (figure 57b,  $r^2 = 94.5\%$ ) and the inaccuracy of the radial dimension (figure 57c,  $r^2 = 47.7\%$ ). Inspection of figure 57c shows that the radial disorder is uniform and not restricted to one portion of the visual field or tectum. Nor is there any sign of error in the choice of A'.

The reason for this pattern of simultaneous order and disorder is not known. It may be that the process generating topography among radial coordinates is not the same as for circumferential; and certainly the latter process is not dependent on the former. However, the most urgent question is whether map R70 represents a stable configuration of visual innervation, or a stage in the development of a fully topographic retinotectal projection like those of R166A and R166B. It is unfortunate that fish R70 died before it could be remapped.



## CHAPTER SEVEN

### RESULTS: VISUOTECTAL MAPS OBTAINED AFTER INTERRUPTED REGENERATION

Seventeen fish were mapped following regeneration in two stages. The first period of regeneration followed complete mid-orbital transection of the optic nerve, and lasted 17, 20 or 33 days. Axons in the dorsal part of the juxta-scleral section of the nerve were then re-cut, with the intention of temporarily interrupting the regeneration of axons growing from central retina. After this lesion, regeneration was allowed to resume for a further period. These fishes' results are labelled with codes of the form "A m D n", where "m" shows the duration of the first period of regeneration, and "n" the second, both measured in days.

The alphanumeric codes are listed:

#### PREFIX

A17D	42	56	72	81	82a	82b
A20D	27	29	30	31	119	129
A33D	61	131	134	146	158	

Few tectal recording sites yielded visually-evoked responses in A20D27 and A20D29. Visuotectal maps from the other fish are shown in figures 64a to 78a. Corresponding scatter diagrams are shown in figures 64b,c to 78b,c.

The results fall into two groups. The following maps (in the first group) appear on visual inspection to be essentially normal: A17D56, A17D82A, A17D82B, A20D129, A33D134 and A33D158. The regression equations and values of  $r^2$  and correlation coefficients for these maps are shown in table 5 and 6.

TABLE 5

REGRESSION EQUATIONS FOR MAPS FOLLOWING  
INTERRUPTED REGENERATION.

(GROUP A)

FISH	figure	CIRCUMFERENTIAL figure b	RADIAL figure c
A17D56	64	$Y = 11.8 + 0.99X$	$Y = 4.81 + 1.04X$
A17D82a	65	$Y = 17.5 + 0.94X$	$Y = 0.27 + 1.19X$
A17D82b	66	$Y = -1.81 + 0.98X$	$Y = 15.5 + 0.94X$
A20D129	67	$Y = -9.41 + 1.06X$	$Y = 6.13 + 1.03X$
A33D134	68	$Y = 1.74 + 1.09X$	$Y = 8.56 + 0.99X$
A33D158	69	$Y = 12.7 + 1.15X$	$Y = 13.8 + 0.87X$

TABLE 6

## MAPS FOLLOWING INTERRUPTED REGENERATION

(GROUP A)

## CIRCUMFERENTIAL DIMENSION

FISH		$r^2$	r	Rank	P	S.E.
A17D56	64b	96.4%	0.98	0.97	<< 0.001	0.005
A17D82a	65b	94.3%	0.97	0.98	<< 0.001	0.011
A17D82b	66b	96.3%	0.98	0.98	<< 0.001	0.007
A20D129	67b	94.4%	0.97	0.98	<< 0.001	0.012
A33D134	68b	93.1%	0.97	0.98	<< 0.001	0.012
A33D158	69b	92.0%	0.96	0.98	<< 0.001	0.016

## RADIAL DIMENSION

		$r^2$	r	Rank	P	S.E.
A17D56	64c	69.1%	0.83	0.87	<< 0.001	0.046
A17D82A	65c	85.6%	0.93	0.92	<< 0.001	0.028
A17D82B	66c	79.9%	0.89	0.94	<< 0.001	0.040
A20D129	67c	86.8%	0.93	0.94	<< 0.001	0.028
A33D134	68c	81.2%	0.90	0.91	<< 0.001	0.033
A33D158	69c	71.1%	0.84	0.83	<< 0.001	0.058

The range of  $r^2$  values for circumferential coordinates (92.0% to 96.4%) is almost identical to that for simple (non-interrupted) regenerated maps (91.0% to 96.8%, excluding R58). The range of  $r^2$  values for radial coordinates (69.1% to 86.8%) is also comparable to the range of  $r^2$  values obtained in simple regeneration (74.1% to 94.1%, excluding R70) and in normal maps (74.8% to 85.4% excluding N1). In addition, the regression equations are similar. The scatter diagrams of this first group of maps are very similar to those produced by uninterrupted regeneration.

#### A17D56

In the radial coordinate of A17D56 (figure 64c) two points (1, 33) are apparently out of line with the others. These are both very close to the vertical part of the tectum, so they are less accurately mapped (see page 74).

A17D56 is notable for the absence of any representation of the upper part of the visual field on the dorsal half tectum (figure 64a). This abnormality has had no discernable effect on the topography of the map.

#### A17D82b

The scatter diagram for the radial dimension of A17D82b has the appearance of a branched line (figure 66c). This is more obvious in the graph of the standard residuals plotted against the tectal coordinates (figure 66d). As with R167 the interpretation of this is partly subjective. It could be argued that topography is simply less precise in the mapping of peripheral visual field (more than  $70^\circ$  from the optic axis). Alternatively, one may conclude that the projection onto medial and caudal tectum is relatively expanded. Supporting the latter interpretation is the fact that the rank correlation coefficient (0.94) is higher than the product-moment coefficient (0.89): in fact only one

map, R166A, has a higher rank correlation coefficient, so by this index the overall topography is very precise.

One may therefore conclude that all the maps in this group are indistinguishable from those obtained after regeneration without the second lesion.

# VISUOTECTAL MAPS OBTAINED FOLLOWING INTERRUPTED REGENERATION

## GROUP B

The remaining maps are distinguished by the fact that one or more electrical responses recorded somewhere in peripheral tectum was evoked by a stimulus in central visual field. These visuotectal maps are shown in figures 70a to 78a, and the corresponding scatter diagrams are in figures 70b,c to 78b,c. Except for this distinguishing feature shared by all the fish, the maps are very varied. They are therefore considered separately rather than en bloc.

A17D42

This map appears essentially normal with the sole exception that the only response detectable at tectal site 5 was evoked by a stimulus in central visual field (5) (figure 70a). In a normal map direction 5 would have been represented by tectum lateral to 12, which images the neighbouring part of visual space, and only the uppermost part of the visual field should be represented at the medial extremity of the tectum.

The response at 5 appeared to be much weaker than all the others, but the amplitude of the visually evoked responses was not quantified. In this map tectum surrounding the abnormal response at 5 was devoid of visual activity.

It is evident from the scatter diagram of the circumferential coordinates (Figure 70b) that the error in 5 is exclusively in the radial coordinate. The value of  $r^2$  for the circumferential dimension is 98.3%, which equals that from N4 as the highest from all the maps presented here.

$r^2$  for the radial coordinates (figure 70c) has the value 53.4%; but if observation 5 is omitted from the analysis this rises to 76.6%, which is comparable to values obtained in simple regenerated maps. This, with the residual plot (figure 70d), confirms that this map is abnormal only

in the isolated defect of having 5 misplaced in the radial dimension.

#### A17D72

For almost all of the recording sites in A17D72 (figure 71a) two local optima for the direction of the visual stimulus were obtained, one in central field, one in peripheral. In every case the central stimulus appeared to evoke a weaker response than the peripheral: consequent difficulty in identifying the optimum stimulus direction may have impaired the accuracy of the mapping process.

The corresponding scatter diagrams are shown in figures 71b and 71c. The circumferential coordinates show close correlation ( $r^2 = 87.4\%$ ;  $r = 0.935$ ). Both central and peripheral groups of numbers fit the same regression line closely. The precision of topography in this dimension is evidently relatively undisturbed in this highly abnormal map.

A simple linear regression in the radial dimension (figure 71c) has the equation  $Y = 33.4 + 0.36X$ . This regression is not significant at the 95% confidence level ( $0.1 > P > 0.05$ ); and this explains only 3.5% of the variation of the visual field coordinates. But it appears that this simple regression is quite inappropriate: two parallel, roughly linear aggregations of points can be discerned, and these are made obvious in the plot of standard residuals against tectal radial coordinates (figure 71d). These two groups correspond to the central and peripheral parts of the visual field, as may be seen in the plot of standard residuals against visual field coordinates (figure 71e). The division of the points by this single regression line is very clear. Points with positive residuals are from peripheral field; those with negative, from central. The only exceptions are points 36 and 38. 38 was recorded as producing a very weak response indeed and its placement may consequently be imprecise. When recording at 36 the multiunit receptive field was found to be very elongated in the radial dimension with no distinct maximum.

Representing this by a single point is therefore misleading. Several of the points with tectal radial coordinates similar to that of 36 were shown to have two optimum stimulus positions within the same radius (e.g. 8, 25, 26, 34). It is plausible to regard 36 as similar to these but with two receptive fields apparently confluent. In many instances in this fish and in others in this group, a visual stimulus position between the identified maxima for a recording site evoked activity above the background level.

The topography in the radial coordinates of central and peripheral groups may be analysed separately. (36 is omitted because of its ambiguity). Considering only those points with residuals greater than or equal to zero in the first, inclusive regression, a new regression equation can be drawn:  $Y = 57.4 + 0.37X$ . ( $a = +57.4$  implies a large central scotoma - see page 74 - and this is obviously correct).  $b = 0.37$  is significant ( $P < 0.01$ ), but this regression line accounts for only 18.7% of the variation among visual field coordinates. The radial coordinates of the central group correlate better: a new regression of the points with negative residuals in the first plot gives the equation  $Y = 5.7 + 0.44X$ . This regression is also significant ( $P < 0.001$ ), and gives a value of 65.6% for  $r^2$ . The correlation amongst circumferential coordinates is also slightly better for the central visual stimuli ( $r^2 = 92.1\%$ ) than for the peripheral ( $r^2 = 86.4\%$ ). Low values of "b" for both of these partial maps indicate radial expansion, which is obvious.

In summary, this map shows two visuotectal maps superimposed over the whole extent of dorsal tectum. One represents central visual field (up to  $37^\circ$  from the optic axis), and the other peripheral. Both are topographic in radial and circumferential coordinates, but not as precisely as a normal map. The circumferential coordinates of the two maps are in register with each other. This extraordinary pattern of tectal innervation has not been reported before.



The obvious abnormality in this map (figure 72a) is that central visual field (up to  $10^\circ$  from the optic axis) is represented twice on the tectum. A strong visually evoked response was recorded at 24, which is the normal site for representation of central visual field. Weak responses were recorded at points 1, 3, 4, 5, 9, 10, 11 when the visual stimulus was close to the optic axis. At these same rostro-medial recording sites stronger responses could be evoked by stimuli in the periphery of the supero-nasal quadrant of the visual field. In the scatter diagrams, weak responses to central visual stimuli are indicated n'.

The scatter diagram of circumferential coordinates (figure 72b) shows that several of the abnormal responses have lower (3') or higher (1', 4', 5', 9', 10') tectal coordinates than would be expected if they conformed to the linear distribution of the normal points. But with the exception of "3" these errors of circumferential coordinate are small, and because all the stimulus directions for these points are close to the optic axis (see page 79), and all the responses are weak, the circumferential coordinates are doubly prone to error. Even when these abnormal responses are included the regression equation ( $Y = 9.96 + 1.09X$ ) is highly significant ( $P \ll 0.001$ ), and the value of  $r^2$  (94.4%) is within the range of the simple regenerated maps. Excluding the erroneous points produces only a modest increment in  $r^2$  to 96.6% ( $Y = 9.66 + 1.09X$ ).

The abnormal responses form a discrete group in the scatter diagram of radial coordinates (figure 72c), clearly separate from the others. The overall regression equation is:  $Y = 15.7 + 0.525X$ . This explains only 13.5% of the variance. This line is significant ( $P < 0.05$ ); but it is obviously inappropriate to draw a linear regression through all of these points. If the abnormal responses are excluded the equation becomes:

$$Y = - 1.70 + 1.08X ; P \ll 0.001, r^2 = 85.9\%.$$

This confirms that the map is normal in the radial dimension with the exception of these identified points.

As in A17D42 and A17D72 malposition of the abnormal responses is almost exclusively in the radial coordinate. The significance of the dual projection from central retina indicated by this map is discussed below.

#### A20D30

This map appears highly disorganized (figure 73a). Several central visual stimulus directions evoked responses in peripheral tectum, rostrally (1, 12), rostro-medially (3, 4, 5), medially (13, 14, 15, 16, 23, 24, 25, 26) and caudo-medially (33). The majority of tectal loci receiving this abnormal input are also innervated by peripheral retina, and their normal peripheral stimuli produce stronger responses than their abnormal central ones. On the scatter diagram of the circumferential coordinates, the more central stimuli are marked n', where they differ from the peripheral ones.

Despite the appearance of the map the correlation in the circumferential coordinate is extremely good (figure 73b):  $r^2 = 91.8\%$ , within the range of values for simple regenerated maps (91.0% to 96.8%). Responses to central visual stimuli (3, 5, 12, 13, 16, 24, 25, 26, 27) tend to be further from the line; but the circumferential coordinates of observations close to the optic axis are always likely to be less accurate (page 79).

The abnormality of pattern is evidently restricted to the radial dimension of topography. The nature of this abnormality is clarified by the scatter diagram (figure 73c) for radial coordinates. As in A17D72 (point 36) a few of the recording sites were found to have multiunit receptive fields greatly elongated in the radial direction: responses could be evoked by stimuli at up to 20° or 30° from the recorded optimum

for loci 1, 9, 10, 11, 21, 32, 36 (bracketed in figure 73c). Another point of resemblance to A17D72 is that for some recording sites the two local optimum stimulus directions had similar circumferential coordinates (e.g. 15, 16, 25, 26): as in the above map it is possible to interpret 1, 9, 10, 11, 21, 32, 36 as examples in which central and peripheral visual fields are contiguous.

With these exceptions the points in figure 73c appear to aggregate in two roughly linear clusters. The central point 20 could belong to either or both lines. Neither the upper line nor the lower indicates very good correlation of coordinates, but the appearance is that of radially topographic maps of peripheral and central field superimposed on the tectum, with similar orientations.

#### A20D31

From the map (figure 74a) it is obvious that points 9, 10, 11, 21, 22 are grossly misplaced, and there is a dual mapping at 2. This map is also abnormal in that the caudal half of the dorsal aspect of the tectum was found to be devoid of visually evoked potentials. It appears that the retinal projection may be displaced over the rostral pole: one would otherwise expect the nasal pole of the visual field to have been mappable.

The scatter diagram for radial coordinates (figure 74c) shows two aggregates of points. In the left and upper parts of the diagram are observations which constitute an approximation to the normal visuotectal map. Points 13, 14 and 25 lie to the right of the long axis of this group; points 29, 30, 31, 32 to the left. This suggests that A' at 18 is misplaced, and better correlation between the radial coordinates of visual field and tectum might have been found if A' were further medial. The subjective choice of A' is made difficult when the central part of the visual field is not obviously topographic; and this is one limitation of the method. One could find the "best" position for A' by trial and

error, revising its position according to the interpretation of the scatter diagram. (This would require the assumption that the map is at least partly topographic). It is already known from A17D42 and A17D81 that such maps as these can be entirely orderly, except for specific observations showing gross errors of topography. It would therefore add nothing to show that the major part of A20D31 can also be made to appear accurately topographic.

The group of six points in the lower right corner of this scatter diagram (2, 9, 10, 11, 21, 22) merely restates what can be seen in the original map: that the central part of the visual field is represented on a broad arc of rostromedial tectum, at one point (2) overlapping the normal projection of peripheral field. Interestingly, as in A17D81, central visual field has a simultaneous normal representation on central tectum, and the visual responses obtained from this appeared considerably stronger than the abnormal responses elicited from the periphery.

Point 1 was recorded as possessing a multiunit receptive field greatly elongated in the radial dimension (cf. A17D72 and A20D30).

The scatter diagram for circumferential coordinates in A20D31 (figure 74b) appears unusual, with abnormal deviations from the expected line in points 16, 17, 18, 19, 22, 28. Of these, all but 22 could be attributable to error in the choice of A' discussed above; and all six have small radial coordinates in the visual field so their circumferential coordinates may be expected to be mapped with less precision than peripheral points.

A20D119

This map is sadly incomplete: the fish died before the end of the mapping experiment. However, the quarter of the tectum which has been mapped (figure 75a) provides another example of central visual field being represented on peripheral (medial) tectum. At two of these points

(2, 4) the normal representation of peripheral visual field could also be detected.

The scatter diagrams for circumferential coordinates (figure 75b) and radial coordinates (figure 75c) indicate good topography among the normal points comparable to that observed in completed visuotectal maps. It appears that the abnormal responses are incorrect in circumferential as well as radial coordinates (cf. 22 in A20D31): but as with all these projections of central visual field it is impossible to know how much of the abnormality is attributable to experimental error during mapping and how much to a real lack of topography among neural connections.

A33D61

This map (figure 76a), like A20D31, shows vacant caudal tectum and a failure to image nasalmost visual field on the dorsal (accessible) part of rostral tectum. (A small arc of rostral tectum was obscured by water: no inference should be drawn about the innervation of this area.)

Point 3 on the tectum received two visual inputs, one from central visual field, one from the normal area of the periphery. A second abnormality in this map is that 2, 3, 6 and 7 have radial coordinates in visual field rather lower than expected. In these examples the multiunit receptive fields were not enlarged (radius up to  $10^\circ$ ), so these points are not in the same speculative category as 1, 9, 10, 11, 21, 32, and 36 in A20D30 and other points discussed above. The scatter diagram also shows that there is a general loss of topographic precision in the radial dimension (figure 76c). This is not attributable to an error in A' and the reason for this appearance is not known. In its widespread inaccuracy this map resembles R70 (figure 57).

The circumferential topography of A33D61 is very good (figure 76b):  
 $r^2 = 90.5\%$ .

A33D131

This fish also died before mapping was completed, but only the temporal extremity of the visual field remained unmapped (figure 77a). Four recording sites in rostromedial tectum (2, 3, 5, 6) were found to have a dual innervation: it was possible to record a normal strong response to a peripheral visual stimulus and an abnormal weak response to a central one. The area of tectum expected to receive the projection from around the optic axis in a normal map was shown to lack visually evoked activity.

The radial coordinates of observation 1 place it in a position intermediate between the normal visuotectal map and the abnormal peripheral representation of central visual field (figure 77a). The multiunit receptive field for this tectal recording site was not enlarged, but interestingly those of points 11 to 20 were.

A33D146

This map (figure 78a) differs from the others reported in this section in that the tectal loci at which an abnormal response to central visual stimulation could be detected were scattered (points 3, 5, 9). Figures 78b and 78c show that this map is otherwise normal.

## CHAPTER EIGHT

### DISCUSSION OF VISUOTECTAL MAPS

#### 1) SUMMARY OF THE MATHEMATICAL ANALYSIS OF VISUOTECTAL MAPS

The salient features of this analysis may be summarized briefly. Polar coordinates (radial and circumferential) are calculated for each tectal recording site on the assumption that the tectum is a spherical structure. (Polar coordinates may also be calculated on the assumption that the tectum is flat: this distortion appears to have little effect on the results.) In each dimension the tectal coordinates are plotted against the corresponding coordinates of the appropriate visual stimulus directions. These scatter diagrams provide a visual display of the topography in each dimension, which proves to be a powerful aid to the analysis of each map. The value of this display is fourfold. Firstly, it allows a ready appreciation of the form of topography: i.e. whether the map is uniform in each coordinate or displays relative expansion or compression of different parts. Secondly, it facilitates the identification of both a general trend of topography and local deviations from that pattern. Thirdly, the breadth of scatter of the points in these diagrams may be used as an indication of the precision of the topography: in a disorderly map the points are widely scattered, whereas in an accurately patterned map they conform closely to a single line. The fourth advantage of this analysis is the segregation of radial and circumferential components of the maps, thereby showing when loss of overall topography is attributable to imprecision in only one of these dimensions. None of this information is unobtainable from the original maps: the benefits enumerated are achieved simply by the clearer presentation of the same data in the manner described.

The elements of subjectivity in this analysis (in selecting the tectal locus (A') most closely corresponding to the optic axis and, in

the three dimensional version, estimating the radius of the model spherical tectum) prove to be unimportant: errors of A' may be detected by a study of residuals, and variation in the radius has little effect on the scatter diagram (page 113).

It is possible to quantify the precision of topography in each dimension using polar coordinates. A formal index of the breadth of scatter in these diagrams (corresponding to imprecision in the map) may be obtained by fitting a regression line to each diagram and calculating a correlation coefficient from this; or by calculating a Rank correlation coefficient. This quantification facilitates comparisons between maps. However, the usefulness of correlation coefficients is limited, firstly because each is adversely affected by points known to be unreliable: it is impossible to measure the circumferential coordinate of a stimulus close to the optic axis with as much precision as that of a peripheral visual stimulus; and in the radial dimension, the few tectal loci close to the vertical part of the tectum are prone to greater errors than those near the horizontal part. Secondly, it is potentially misleading to compare the precision of radial and circumferential dimensions because the accuracy with which individual coordinates are mapped is not necessarily the same. (See p.105) (The latter is a theoretical reservation: no reasons for systematic error of mapping in the radial dimension is yet known). Conclusions drawn from these coefficients must always be qualified by reference to the scatter plots, and with due attention to the "if's and but's" discussed in chapters 5 and 6.

## 2) NORMAL VISUOTECTAL MAPS

The results demonstrate quantitatively and unequivocally the high precision in both of the polar dimensions. This is an improvement on the statistical test devised by Gaze and Jacobson (1963) (discussed on page 68) in which it was only possible to calculate the statistical



significance of apparent orderliness (see page 68).

The other achievement of the present method is to show the striking uniformity within nearly all of the visuotectal maps: there is no relative expansion or compression of any part of the visual field. The visuotectal map may in this sense be described as homogeneous as well as topographic. (This is functional uniformity: if ganglion cells are evenly distributed across the retina of the goldfish, as indicated by Johns and Easter (1977) and by Ioannides (cited in Jacobson and Gaze, 1964), one must conclude that there is also anatomical uniformity among the axons' terminal arborizations. In certain other teleosts, ganglion cell distribution is far from uniform (Ito and Murakami, 1984): it would be interesting to assess the uniformity of representation of the visual field on the tectum in these fish.) Jacobson and Gaze (1964) calculated a "magnification factor", defined as the number of microns measured linearly across the tectum representing one degree measured meridionally in the visual field. In normal goldfish this varied between 8 and 20  $\mu\text{m}/\text{degree}$  for different parts of the field; but there was no systematic variation and no sign of an area centralis.

It could be that individual axons show little variability in the extent of their arborization because of factors intrinsic to each ganglion cell; or there could be competition between fibre terminals for synaptic sites. Competition between the elements of the retinotectal projection may explain the compression and expansion of retinotectal projections (Gaze and Sharma, 1970; Horder, 1971; Straznicky, Gaze and Keating, 1971; Yoon, 1972). It has also been invoked in various models postulated to control topography (Prestige and Willshaw, 1975; Willshaw and von der Malsburg 1976; Hope, Hammond and Gaze 1976); but the demonstration of uniformity has no bearing on whether topography per se is generated in the manner proposed by those models.

Evidence of non-uniformity in retinotectal projections distorted by

secondary expansion or compression indicates that uniformity is not an invariant accompaniment to the development of topography; but one cannot exclude the possibility that projections in which non-uniformity was indicated by measurement of "magnification factors" (Schmidt, Cicerone and Easter, 1978) or by inspection of electrophysiological (Meyer, 1977) and autoradiographic (Meyer, 1980) maps were still undergoing a process of reorganisation involving competition between optic axons. This may explain the discrepancy between the results referred to and the report (lacking quantitative support) that compressed retinotectal projections may be uniform (Cook and Horder, 1974).

There are also contradictory reports about the uniformity of the expanded projections formed by the component halves of compound retinae. Gaze, Jacobson and Szekely (1963) found that the projections of double-nasal and double-temporal retinae are evenly distributed. But Straznicky, Gaze and Keating (1974) showed relative expansion of the central part of the double-ventral projection. Studying the progressive spread of compound eyes' projections by autoradiography, Straznicky, Gaze and Keating (1981) found that uniform density of innervation was never achieved. It may be that competition results in uniform allocation of tectal space only when there is an excess of fibres in search of termination sites. Formal assessment of the uniformity of the visuotectal map whose fibre complement was only 5% of the normal (Springer et al., 1977) would clarify this.

In future an additional benefit is likely to accrue from the application to normal maps of the mathematical analysis described here: if the orientation of the eye is accurately controlled during the mapping experiment it will be possible to infer (from the first constant ("a") in the regression equation drawn through points on the scatter diagram of circumferential coordinates) how accurately the orientation of the retinotectal projection is controlled.

### 3) MAPS OBTAINED AFTER SIMPLE REGENERATION

The results presented above show quantitatively that as a result of regeneration both circumferential and radial topography can be restored to a level of precision as great as in the original retinotectal projection (at least after 166 days of regeneration, under these conditions). This is not surprising: for many years it has been obvious from inspection of visuotectal maps without quantification (e.g. Gaze, 1970) that the accuracy of overall topography appears to be comparable in regenerated and normal projections.

By providing a quantitative verification of this the present results highlight the fact that the processes generating topography are independent of the numerous differences between conditions prevailing in development and in regeneration, such as the micro-environment through which axons grow to the tectum, and the area of vacant tectum available to the first-regenerating fibres. By demonstrating this, these results accentuate the need to discover how accurately the radial sequence of axonal arrival in regeneration replicates that of development. It may be less precise in regeneration than in development, simply because in development this process is very gradual, occurring as long as the fish's eye grows, whereas in regeneration the entire radial extent of the retina reconnects with the tectum in a few weeks. Further information about this is necessary if one is to evaluate the extent to which sequential arrival of axons could be responsible for radial topography.

It has been suggested that abnormalities in the visuotectal map of R58 might reflect immaturity of this projection. It would be interesting to follow up this observation, inquiring whether visuotectal maps obtained after brief periods of regeneration consistently show both elongated multiunit receptive fields and a few misplaced elements. Similar receptive fields have been reported by Meyer (1977) during the

early stages of compression. Among the maps obtained after interrupted regeneration, there is a preponderance of markedly abnormal maps after short periods (A17D-42/72/81; A20D-30/31; A33D61); but the number of fish is small, so this does little to support the theory of progressive refinement.

Schmidt and Edwards (1983) have reported that the multi-unit receptive field size in visuotectal mapping decreases from  $30^\circ$  to  $10^\circ$  during the maturation of regenerated projections; and the present data are compatible with that result. But it could be that this change reflects shrinkage of overlapping axonal arborizations, without alteration of the mean positions of the axons' terminals, which is the true denominator of topography. Quantitative estimation of the precision of topography in the manner described here would therefore be more informative. The ideal experiment would repeatedly map individual fish at, for example, 20, 40, 60, and 80 days of regeneration. It might also be advantageous to resolve topography into radial and circumferential dimensions, which the measurement of multiunit receptive field sizes does not do.

There is also uncertainty about changes in the precision of the retinotectal projection during development. In Xenopus laevis, Gaze, Keating and Chung (1974) found that the first visually-evoked responses could be recorded at embryonic stages 43 to 44 (Nieuwkoop and Faber, 1956), and that the map was initially disorganized. But using an anatomical technique for the dorso-ventral axis of the tectum and electrophysiology for the rostro-caudal, Holt and Harris (1983) have shown topography from stage 39, and no prior phase of disorder.

The time-course of the formation or reformation of the map may clarify the nature of the organizing processes. If topography is present ab initio during regeneration and in development, this would imply that the pattern-forming events occur either during the growth of the axons

towards the tectum or immediately upon their arrival at that target.

If topography evolves gradually in situ, one must conclude that the optic terminals are dynamic rather than static, capable of interacting to generate order amongst themselves (as in the theoretical model invented by Willshaw and von der Malsburg (1976)). This is quite plausible: it is already known that during development the retinal projection shifts caudally across the tectum (Straznicky and Gaze, 1971; Johns, 1977; Cook, Rankin and Stevens, 1983; Easter and Stuermer, 1984), and this might possibly require a mechanism which actively sustains topography against disruption caused by movement. If the development of accurate radial topography is a delayed phenomenon, sequential arrival of axons cannot be a sufficient explanation for the final precision of this dimension of the map; although it may still contribute if initially there is imprecision rather than absence of order. Indeed, for certain models of active sorting of axon terminals such a contribution might be essential (see chapter 11).

Experiments designed to clarify the time of organization of the regenerating retinotectal projection have been performed in this laboratory subsequent to the work described here (using a two dimensional model tectum). Preliminary results show a progressive increase in the accuracy of the radial dimension of topography as the duration of regeneration increased from 21 to 120 days (at 15 to 20 C) (Sharma, A.K., 1984).

Recently, new anatomical evidence about the refinement of the retinotectal projection has been reported in summary by Rankin and Cook (1984). Iontophoresis of a small volume of HRP into the tectum labels a small cluster of ganglion cells in the retina, if the optic projection is normal. After 28 days of regeneration, labelled cells were scattered across the appropriate quadrant of retina, and were occasionally found elsewhere: there was no local cluster. At 42 days, large clusters of

cells were found, and these were often extended along the radius of the retina. After 56 days, clusters were still enlarged; two retinae each contained two irregular aggregates of cells, loosely linked along a radius. Subsequently clusters reduced towards the normal size. The difference between the refinement of the circumferential dimension and the relative disorder of the radial is most interesting: these findings may be regarded as an anatomical counterpart to the visuotectal maps presented here, in which radial order is noticeably impaired (best seen in R70: figure 57a,b,c).

#### 4) THE EFFECTS OF INTERRUPTING REGENERATION

Two distinct categories of results were found. Six maps (group A) were entirely normal; nine maps (group B) were very varied, but shared the common attribute of containing abnormal responses weakly evoked in peripheral tectum by central visual stimuli. In interpreting these diverse results it is necessary to consider all the possible effects of resectioning the fibres from central retina during or immediately after their regeneration.

The first possibility is that the second lesion results in mechanical constraints being put upon these fibres, such that when they regenerate anew they are unable to re-occupy their original synaptic sites - perhaps because of obstruction by other fibres given precedence. Several fish were examined macroscopically post mortem: no gross abnormalities of the optic tract were seen, but the precise course of fibres from central retina could not be identified. In *Xenopus* regenerating fibres grow over the surface of the diencephalon rather than follow their original paths (Gaze and Grant, 1978). If a similar phenomenon occurred in goldfish, resectioned axons from central retina growing superficially over fibres of peripheral retina might have no access to the central part of the tectum: the influence of sequential

arrival of axons in limiting their termination sites would still be genuine, but would be indirect. However, goldfish appear to differ from *Xenopus* in this respect: following a cut through the optic nerve regenerating axons from central retina are dispersed throughout the optic tract (Dawney, 1981a). Because regeneration in these animals still achieves radial topography of connections (Dawney, 1981b), the position of an axon in the tract must be irrelevant to this aspect of its termination site. Mechanical effects of the second lesion in these experiments therefore cannot be blamed for the abnormality in the maps.

Another possibility is that the second lesion leads to some disruption in a chemical labelling system, upon which the radial topography could depend. Without knowing that there is such a system, and having no idea of its nature, this suggestion is entirely speculative. The obligation to consider it arises from previous theorizing (Sperry, 1963) not from the results themselves. Repeated crushing of the optic nerve at weekly intervals for up to thirteen weeks was followed (six months after the last axotomy) by demonstration of a map not noticeably different from those formed after single regeneration (Horder, 1974a). If a cytochemical labelling system does exist it does not seem to be affected by axotomy, so this explanation of the present results may be rejected. The abnormalities of these retinotectal projections may therefore be attributed to the third possible effect of the second lesion, which is a disruption of the usual centrifugal sequence of arrival in subsequent regeneration.

The fact that the circumferential dimension of topography is undisturbed by the second lesion proves that the effect of interrupting regeneration is a selective disturbance of radial order, not a generalized loss of precision. Moreover, the nature of the disturbance achieved is exactly in accordance with expectations derived from the theory that sequential arrival of axons regulates the radial coordinates

of their terminations; and with the prediction that in some of these fish the regeneration of axons from central retina would be delayed.

It was not possible to demonstrate the sequence of arrival of regenerating axons in these experiments: repeated visuotectal mapping might be one method of achieving this; but it proved impossible to keep these fish alive for long periods after the mapping experiments. However, the validity of this as a means of investigating the time-course of reinnervation depends on various accessory assumptions about the electrical detectability of newly grown axons (see page 53). The conflict of results between Holt and Harris (1983) and Gaze, Keating and Chung (1974) suggests that subtle differences in recording technique might be critically important in the use of electrophysiology for anatomical mapping. Further examination of the technical variables is still required.

Anatomical methods for demonstrating the sequence of arrival of axons (as in chapter 3) require the sacrifice of each fish, preventing investigation of the topography of subsequent connections.

Variation between fish in their rates of regeneration (evidenced by the first experiment and previously documented by Springer and Agranoff, 1977) makes it unreliable to extrapolate from fish studied anatomically (in the former experiment) to draw conclusions about the state of regeneration in other fish to be mapped later on. Because this was the only available cue about when to re-section the regenerating fibres, the partial lesions of the optic nerve were performed, unfortunately but necessarily, without proof of any disruption of the sequence of arrival of optic axons at the tectum. For this reason the second lesions were performed at a variety of intervals after the first, in the hope that in some fish the timing would be opportune. This is one reason for the variation between maps: but differences between fish in the rate of regeneration, the length of the optic pathway, the exact positioning of



the lesions, and variation in the extent of the second lesion are also likely to contribute.

Uncertainty about the sequence of arrival of regenerating axons is compounded by the fact that axons regenerate faster after a second transection (Lieberman, 1974; McQuarrie and Grafstein, 1981), and after a lesion which is closer to the perikaryon (Watson, 1968).

For these reasons a normal visuotectal map constitutes a "weak" experimental result and less significance should be attached to the six fish showing this pattern.

The feature held in common by the nine fish of the second group is that electrical responses evoked by stimuli in central visual field could be recorded in peripheral tectum. This striking abnormality is entirely consistent with the idea that the sequence of arrival of regenerating axons restricts the radial coordinates of their termination sites: if the regeneration of axons from central ganglion cells is delayed by the second lesion they may arrive at the tectum after, or in synchrony with, axons from the periphery which normally follow them, and therefore terminate further peripherally, or superimposed upon the others.

Is this too simplistic an interpretation? In six maps (A17D42; A17D81; A20D30; A20D31; A33D61; A33D146) there is contradictory evidence: central retina apparently connects with central and peripheral tectum. It is impossible to know whether individual ganglion cells connect in both places simultaneously. It seems unlikely that some of the axons in the dorsal part of the nerve should have remained uncut: although the precision of topography within the dorsal part of the juxta-scleral segment of the optic nerve has not been directly demonstrated in these particular animals, the central group of ganglion cells marked by HRP applied to such lesions in other animals does not appear to contain unlabelled cells. Confirmation of this expectation would have been useful; but it was not possible to use HRP to identify the ganglion cells

whose axons were cut by the second lesion, because this enzyme can not be relied upon to last long enough in these cells for it to be detected after the mapping experiment. The reason for these double central retinal projections is therefore not known.

This remains an important problem: one possible explanation is that axons from central retina may generally elect to terminate in central tectum (or in the central part of the presynaptic array) after recognising specific positional cues. Without re-examination of the fish with misplaced central retinal projections, one cannot prove that these errors are not transitory phenomena subsequently abolished by active re-organization.

#### 5) THE ABNORMAL WEAKNESS OF MISPLACED VISUOTECTAL RESPONSES

All the abnormally placed responses indicating a projection from central retina to peripheral tectum were judged subjectively to be very much weaker than responses obtained in the remainder of the map, or from any part of a normal map.

The weakness of the abnormal responses found here raises a suspicion that they do not arise from the terminals of optic fibres. It would be helpful to have anatomical evidence of a misplaced projection from central retina, or to have demonstrably post-synaptic recordings showing the same result. Even behavioural evidence of misdirected visuomotor reflexes would support the identification of the weak responses reported here. But in the absence of such direct evidence one can still argue against the possibility that the recordings reflect activity in fibres passing through but not stopping in peripheral tectum: similar fibres of passage would have been expected after uninterrupted regeneration, but weak, abnormally sited responses were not obtained.

Maps A17D72 and A20D30 provide the firmest evidence that these weak responses do represent retinotectal axon terminals, because their

topography could not be produced in any other way. The weakness of these responses therefore does not prohibit interpretation of their positions.

There are numerous possible explanations for a weakness of optic nerve terminal activity. Firstly, there could simply be fewer axons present. Axotomy commonly results in some cell death: proximal axotomy may kill more cells than distal (Lieberman, 1974), and repetition of the insult may further reduce the number of cells left. Ganglion cell loss in the fish mapped in this experiment was not measured: stained with cresyl violet, their somata appeared swollen, and could not always be distinguished from each other.

Another possible reason for the weakness of the abnormally placed responses is that the second lesion could result in long-lasting damage to central retina, such that central visual stimulation is no longer as potent in evoking ganglion cell activity. However, normal strength responses were recorded from central tectum following central visual stimulation in some of the fish in which weak activity was also elicited peripherally, so it is not likely that the fault lies in the retina.

The misplaced projections may be comparatively immature if these axons' regeneration was delayed. But at more than 100 days after the second lesion this is not likely to be the reason for the weakness of responses, since strong responses can be recorded after much shorter periods from axons which have regenerated to their normal tectal sites.

A fourth possibility is that the nature of the connections which optic axons are able to form might depend on their positions (within the presynaptic array, rather than with respect to the tectum). For example, it has been suggested (Willshaw and von der Malsburg, 1976) that there could be a mechanism for reinforcing correctly placed temporary connections. In the model proposed by these authors, incorrectly placed initial contacts (which need not be fully-formed synapses) were presumed to disappear. A more common but equivalent postulate is that errors of

projection are corrected post hoc by withdrawal of axons (Purves and Lichtman, 1980). This has been demonstrated in the developing retinotectal projection of the chick (McLoon, 1982; Fujisawa, Thanos and Schwarz, 1984), where it is accompanied by a marked reduction in the number of optic fibres (Rager and Rager, 1978). Similar reduction occurs in the regenerating retinotectal projection of the goldfish (Murray and Edwards, 1982). But one cannot assume that all erroneous terminations are physically removed: possibly some persist for months with reduced activity. This could be important in making the overall projection function as though topographic even when some individual fibres are misplaced; and this suggestion should be considered as a possible adjunct to mechanisms concerned with anatomical order.

#### 6) SUPERIMPOSITION OF PARTIAL VISUOTECTAL MAPS

The map of A17D72 is the most important of the results. It shows that central and peripheral regions of the visual field are represented simultaneously over the tectum. A20D30 shows a very similar result, with duplication of representation, although this is not so complete.

Superimposed maps have previously been described in various experimental situations. Firstly, they occur when two different retinae are surgically induced to innervate the same tectum simultaneously, by the diversion of one contralateral projection on to the ipsilateral tectum (Hunt, 1977; Schmidt, 1978). Secondly, the ipsilateral projection which is normally present in *Xenopus* may regenerate to the contralateral tectum instead, producing partial duplication of the tectal innervation (Gaze and Jacobson, 1963; Gaze and Keating, 1970). Thirdly, the two halves of surgically created compound eyes can form superimposed projections (Straznicky, Gaze & Keating, 1974), as can eyes whose embryonic rudiments were simply bisected and left in place (Hunt and Jacobson, 1974b; MacDonald, 1978). A map of one half of the visual field may also

be superimposed with reversed nasotemporal polarity on top of a map of the other half field which has normal orientation (Martin, 1978). Chung and Cooke (1978) have shown that excision, rotation and reimplantation of embryonic neural tube tissue in *Xenopus* can also result in a projection with reversed naso-temporal polarity being superimposed on a normal projection. The present report is the first description of two concentric projections from the same retina being superimposed on the same tectal area.

In A17D72 the centres of the two partial maps are approximately coincident and both maps span the full radial extent of the tectum. Both A17D72 and A20D30 vindicate the view that retinotopy in the radial dimension is intrinsic to the fibre array and even to a subset of the fibres of the projection, and is not dependent on the occupation by the axons of a particular locality on the tectum. Furthermore, if one assumes that the effect of interrupting regeneration in the manner described is to delay the reinnervation of the tectum by axons from central retina, the obvious interpretation of these maps is that central and peripheral groups of axons have reinvaded the tectum concurrently. This is consistent with the conclusion that a central-to-peripheral sequence of arrival of regenerating axons is a pre-requisite for the establishment of a normal coherent, single map. However, these maps do not show that timing is responsible for the precision of radial topography within these subsets of the map.

All the weak, abnormally-placed responses (except those of A33D146) show negligible error of circumferential coordinates. In A17D72 this means that the two part-maps are superimposed with the same orientation. Two interpretations are possible: they could be oriented independently by a common mechanism, or there could be interaction between the two populations of fibres, manipulating each other's orientation.

## 7) POLAR COORDINATE ANALYSIS

The maps showing superimposition of partial projections provide the most vivid illustration of the merit of examining topography in terms of polar coordinates: Cartesian geometry would conceal the accuracy of correspondence of circumferential coordinates. The same is true of all the other fish in which interruption of regeneration resulted in disturbance of the visuotectal map, for in each case the abnormality is only in the radial dimension, and circumferential topography is accurate.

Polar analysis is likely to be important in future investigation of the mechanisms responsible for retinotopy: if circumferential and radial order are separable, one may infer that the organising processes probably operate in these dimensions rather than in Cartesian axes, or as an indivisible two-dimensional entity. This argument is considered at greater length in chapter 11. One consequence of this suggestion is important here, for it explains the purpose of the following experiment.

It is argued in the introductory chapter that topography is an intrinsic property of the set of optic axons, not a pattern imposed by the tectum; and that "passive" and "active" models must both be considered in seeking to understand the origin of this pattern. The "passive" model of mutual contact guidance of axons has previously been investigated by examination of the paths taken by axons on their way to the tectum. The normal optic pathway is retinotopic in the nerve and tract (Dawney, 1979a); but when the optic fibres regenerate after a mid-orbital cut through the optic nerve their arrangement in the optic tract ceases to bear any relationship to the radial coordinates of their cell bodies, and there is no re-segregation of fibres according to this dimension during their path back to the tectum (Dawney, 1981a; Cook, 1983). This excludes mutual contact guidance of axons as an explanation of the topography of axon terminals; but only in respect of the radial dimension.

The normal optic tract in goldfish is also retinotopic in the circumferential dimension, with fibres from temporal retina occupying adjacent parts of the two brachia, and fibres from nasal retina at dorso-medial and ventro-lateral extremities of the tract (Dawnay, 1979a, 1981b). However, this pattern of topography is not consistent with that in the optic nerve (Dawnay, 1979a) and the transformation from nerve to tract must involve some loss of neighbour relationships. The extent to which contact guidance of axons can be responsible for the circumferential dimension of the normal retinotectal projection is inversely related to the extent of this disorganisation. Studying a similar situation in the Cichlid "Dempsey" fish, Scholes (1979) proposed a theoretical model in which loss of topography is kept to a minimum by the division of the optic nerve into four bundles, each of which remains internally ordered. These are proposed to exchange places and rotate about their own axes as they traverse the region of the chiasm. There is no experimental support for this. Bunt (1982) described an appearance of interdigitation of many fascicles of fibres when studied in longitudinal sections; but this does not adequately differentiate between quadrantic retention of order in subsets of axons behaving as Scholes imagined, and gross disruption to the extent that contact guidance can contribute nothing to the pattern of connections. If the nature of the transformation between nerve and tract is to be understood it must be studied with special reference to the circumferential dimension.

Results of such analysis will be significant whatever they are. If they show that contact guidance could contribute to circumferential topography in the normal projection, it will be necessary to disrupt completely neighbour relationships and to assess the effect of this on visuotectal mapping (cf. Dawnay, 1981b, with respect to radial topography).

If circumferential topography is entirely lost in the normal animal

the presence of circumferential retinotopy in the normal optic tract indicates the existence of a mechanism resegregating fibres. It would then be most important to discover whether tract topography is a direct consequence of fibre interactions operating within the optic tract, which results in the active selection of retinotopically appropriate neighbours, or whether tract topography is an artefact, produced by the combination of being fixed in a topographic array at the tectal end and of being stretched during the growth of the fish. This question can also be answered by studying the circumferential topography of the regenerated optic tract, for there is negligible growth of the fish during reformation of an optic projection. The presence of circumferential topography in the regenerated optic tract (but not closer to the eye) must be due to active recognition and selection of appropriate neighbours; absence of this pattern would demonstrate that the organisation of optic axons requires some process occurring only on the tectum itself, when the behaviour of individual axons changes from growth to synaptogenesis.

The following experiment therefore addresses the questions: (1) to what extent are circumferential neighbour relationships preserved throughout the optic pathway in development and in regeneration? And (2) do optic axons display any propensity to resegment en route for the tectum according to the circumferential coordinates of their retinal origins?



## SUMMARY OF SECOND EXPERIMENT AND OF MATHEMATICAL ANALYSIS

The quantitative analysis of normal and regenerated visuotectal maps demonstrates (1) precise topography; (2) precision greater in the circumferential than the radial dimension of the map without correlation between the two; (3) that radial topography may be imprecise without diminution of circumferential orderliness.

The method described will be essential for investigation of the possibility that mechanisms responsible for topography operate in these polar dimensions. It will also permit quantitative analysis of the control of the orientation of the projection.

Visuotectal mapping after an attempt to alter the sequence of optic fibre regeneration yielded inconsistent results. New forms of abnormal projection were demonstrated: some showed fragmentation of the map in a manner consistent with the suggestion that sequential arrival of axons can contribute to the radial distribution of their terminals. Better techniques must be introduced to permit definitive studies.

The third experiment investigates mechanisms which might contribute to topography in the circumferential dimension, as outlined on pages 146 - 147.

## CHAPTER NINE

### RESULTS :

#### ANTEROGRADE TRACING OF AXONS ORIGINATING FROM NARROW SECTORS OF RETINA

Sectorial applications of Horseradish Peroxidase to the retinas of  
(page 40)  
thirteen fish produced interpretable results. In eight of these the optic  
pathway was normal; in the other five the optic nerve had previously been  
cut in mid-orbit and allowed to regenerate for 104 to 106 days. The HRP  
applications were made on various aspects of the eyes: of the normal  
fish, two had dorsal applications, one dorso-nasal, two ventro-nasal,  
two ventral, and one ventro-temporal. In the "regenerated" group five  
positions (dorso-temporal, dorsal, dorso-nasal, ventro-nasal, ventral)  
are represented by one example each.

Ganglion cells and axons labelled with HRP were identified  
microscopically by the presence of the characteristic pigmentation in  
their cytoplasm resulting from the HRP/DAB reaction. Drawings of the  
retinas (flat-mounted, figures 79 to 91) show the positions of the HRP-  
labelled ganglion cells (dots) (or axons (lines) in two cases) in  
relation to the perimeter of the retina, the optic nerve head, and the  
surgical lesion.

Each retina is followed by drawings of selected transverse sections  
of the corresponding optic pathway (magnification approximately  $\times 100$ ).  
These show the positions of HRP-filled axons as short lines. The relative  
positions of the transverse sections are shown in the diagram below each  
drawing, by the following convention: the horizontal bar represents the  
straightened optic pathway standardized to the same length in each case.  
"R" indicates the retinal end, "T" the tectal, and "X" the approximate  
position of the chiasm, identified by the marked reduction in the cross-  
sectional area of the pathway at this point, and the dorso-ventral  
flattening where right and left pathways cross each other. Vertical

strokes indicate the relative positions of the drawn sections, with the long, numbered stroke referring to the drawing immediately above that diagram. This convention simplifies the comparison of different-sized fish, and fish whose optic pathways might have shrunk to different extents in the histological fixation. The correct orientation (dorsal uppermost) has been preserved throughout; the sections are drawn as seen looking towards the tectum.

Neither the dots representing cell bodies nor the lines representing axons are drawn to scale: only their positions are accurate.

#### RETINAS

The drawings of retinas show that in these fish the method of applying HRP is capable of producing a narrow sector of labelled cells which in most cases is almost complete, extending continuously from the lesion near the papilla to the perimeter. A greater number of applications were unsuccessful and have been omitted because anterograde labelling with HRP was inadequate (which may be the consequence of failing to secure the HRP to the retinal lesion); or because too wide a sector was labelled.

In all but two of the retinas presented here, HRP-labelled ganglion cell bodies could readily be identified by the brown pigmentation produced by HRP/DAB histochemistry. In contrast to their granular appearance after application of HRP to the optic tract, cells labelled by intra-retinal application of enzyme were uniformly stained (plate 5). It may be that HRP reaches these perikarya by diffusion rather than by vesicular transport ((LaVail and LaVail, 1974); and perhaps the shorter survival period following enzyme-application has not allowed HRP to enter lysosomal vesicles.

HRP-stained axons in the retina were well-defined both as a discrete bundle between the lesion and the fundus (plate 7) and as a wedge

converging from the labelled cell bodies on to the lesion (plate 8). In many cases labelled axons and cell bodies were seen to be in continuity (plate 6). In others the proximal parts of the axons could not be distinguished, but the sector of axons and the sector of cell bodies always coincided so the origin of such axons is not in doubt. In the eleven cases in which stained perikarya could be seen the corresponding axons have been omitted from the drawings.

In two fish no labelled ganglion cell bodies could be seen but labelled axons were visible. In one case (regenerated, dorso-temporal sector) these fibres could be seen peripheral to the lesion (figure 87); in the other (normal, dorsal sector 2) they are visible only between the optic papilla and the lesion (figure 80). In both instances the bundle of fibres entering the optic nerve is a discrete group just as in all the examples in which perikarya are also labelled. It therefore seems reasonable to assume that these also represent sectorial applications.

There is some variation in the sizes of the sectors of labelled cells, reflecting some variation in the lesions' sizes and distances from the papilla.

The population densities of labelled cells within their various sectors also appears to differ between retinae. Unlabelled ganglion cells were not counterstained. It is possible that the method of application of HRP fails to label some of the ganglion cells present. This does not invalidate the assumption that HRP-labelled axons originate from the sector containing labelled cells.

#### EXTRA-OCULAR OPTIC PATHWAYS

##### a) GENERAL REMARKS

Some of the axons leaving the eye are very clearly labelled with HRP, being filled by brown reaction product, and are readily identified as far caudally as the tectum, especially in normal pathways. Using light

microscopy it was not possible to follow them into the termination layer.

In all cases fewer labelled fibres could be seen in sections further from the eye.

The definition of retinotopy in the optic pathway is that neighbour relationships between axons should be the same as those of the ganglion cell bodies from which the axons stem. This does not require that a pattern of cells be imitated by a geometrically congruent or similar pattern of axons (see pages 76 to 79): axons from a sector of ganglion cells need not be sectorially arranged in the optic nerve or tract. Nor are there any prima facie reasons for expecting fibres from a particular sector to course through a specific sector or segment of the section; nor for predicting a certain pattern of clustering of axons. The present method of labelling does not guarantee to mark the axons of all the cells in a sector, but it is clear that the greater the preservation of neighbour relationships, the closer together will be the labelled axons. This is the only legitimate assumption about the distribution of labelled axons.

In some sections (especially those containing few labelled axons) it is necessary to consider whether the distribution of fibres could occur merely by chance. A simple statistical test is used to find the probability of this.

The method used here is as follows. A computer graphics tablet is used to find the total length of the shortest branching line which will connect the centres of all the labelled axons. The mean and standard deviation of interaxonal distances are calculated. The same procedure is repeated for the same number of points (representing axons) distributed randomly within the perimeter of the selected transverse section. For this, random distributions are created using a random number generator for x and y coordinates ("Minitab" statistics package, Imperial College Computer Centre). This procedure is repeated on ten different random

plots to find the mean separation which would have been expected had the same number of axons been randomly distributed. The "t" test is then used to find the probability (P) that the observed mean separation could occur in a sample from a randomly distributed population of axons.

This test was applied to sections where there was doubt about the significance of apparent aggregation of axons. The results will be considered in a retinofugal sequence for normal and then regenerated pathways.

#### b) OPTIC NERVE HEAD

In eight of the fish the labelling of a single sector of ganglion cells is reflected in the appearance of a single, more or less coherent group of axons in the proximal part of the optic nerve (section 1 in each of figures 80, 81, 83, 85, 86, 87, 88, 91). But in the other five (three normal (dorsal, ventro-nasal and ventral, figures 79, 82, 84, first sections) and two regenerated (dorso nasal and ventro-nasal, figures 89 and 90, first sections)) there are two distinct groups of axons in the nerve, even though only a single sector of ganglion cell bodies and only a single sector of intra-ocular axons has been labelled.

#### c) NORMAL FISH : OPTIC NERVE AND CHIASM

Without exception these fish show that axons which are very close together in the proximal part of the nerve become much more widely dispersed in cross-sections of the distal part of the nerve and the chiasm. The "t" test described above was applied to a section from each fish, taken through, or close to, the chiasm. The result, expressed as the probability (P) that the observed distribution of axons would arise by chance in a population of randomly dispersed axons, is very variable: see table.

RETINAL SECTOR	FIGURE	SECTION NUMBER	P
Dorsal	79	4	$< 0.001$
Dorsal	80	5	$< 0.02$
Dorso-nasal	81	6	$0.1 > P > 0.05$
Ventro-nasal	82	4	$< 0.001$
Ventro-nasal	83	4	$0.5 > P > 0.1$
Ventral	84	5	$< 0.001$
Ventral	85	8	$> 0.5$
Ventro-temporal	86	4	$0.5 > P > 0.1$

But it is obvious from simple inspection of the pictures to which these figures relate that in all, even those in which the axons are clearly not randomly dispersed, the axons are distributed throughout a proportion of the cross-sectional area which is commonly more than half, and certainly very much greater than the proportion of the retina occupied by the corresponding cell bodies. This implies a substantial overlap and intermingling of different retinal circumferential coordinate values, and consequently a loss of neighbour relationships.

#### d) NORMAL FISH : OPTIC TRACT

In seven fish the labelled fibres could be followed beyond the chiasm. In every section the axons appeared less heavily stained than in sections nearer the eye, but it was always adequate for confident identification of them. However, the density of staining within the sections was variable, and the axons could not be followed through serial sections in every fish. In the fish with a dorsonasal sector of retina labelled, no fibres could be identified more caudal than the chiasm.

In every fish the caudal sections show that the labelled axons are restricted to a small part of the cross-section. Re-application of the "t" test to selected sections showed that it is most unlikely that the

observed patterns of fibre distribution could have arisen in samples taken from randomly dispersed populations of axons (see table).

RETINAL SECTOR	FIGURE	SECTION	P
Dorsal	79	5	< 0.001
Dorsal	80	8	< 0.001
Ventro-nasal	82	7	< 0.001
Ventro-nasal	83	5	< 0.05
Ventral	84	9	< 0.001
Ventral	85	10	< 0.001
Ventro-temporal	86	6	< 0.01

It therefore appears that there is an increase in orderliness according to the axons' circumferential coordinate of origin, in the few hundred micrometers between the chiasm and these more caudal sections.

The positions of the groups of axons within the cross-sections are irrelevant to an assessment of orderliness; but since the material presented has some interesting features in this respect, it may be described briefly. In both fish with ventral applications of HRP the labelled fibres all enter the dorso-medial brachium of the optic tract - which is considered to be "normal". But in the first of the fish with a dorsal sector of retina labelled, a single stray fibre passes into this dorso-medial brachium instead of the appropriate ventro-lateral one (figure 79, section 7). This axon could not be traced retrogradely to the proximal part of the optic nerve so one cannot know whether this fibre came from a group of axons different to that occupied by all the axons in the "correct" brachium. Nor can it be traced far anterogradely, so one cannot prove that it shared the same destination as the others.

In the second dorsally-labelled fish a single coherent group of



axons in the optic tract divides and approximately equal numbers enter each brachium (figure 80, section 9). (In this fish there was only a single group of fibres in the proximal part of the nerve.)

#### e) REGENERATED OPTIC PATHWAYS

In all five cases the circumferential dimension of retinotopy was shown to be completely disrupted by the mid-orbital transection of the nerve:

RETINAL SECTOR	SECTION		
Dorso-temporal	87	3	( Observed mean axon separation is the same as in the random point plots.
Dorsal	88	8	
Dorsonasal	89	8	
Ventro-nasal	90	7	
Ventral	91	6	P > 0.5

Lines resembling fine, HRP - stained axons could be discerned in selected sections of the regenerated optic tracts; but independent observers did not concur about the identification of these, so these sections have been omitted.

## CHAPTER TEN

### DISCUSSION OF RESULTS OF ANTEROGRADE AXON TRACING EXPERIMENTS

#### 1) TRACING OF AXONS

The success of this study depended on being able to mark a cohort of fibres from a narrow sector of retina, and being able to trace them through the optic pathway. The first requirement is fulfilled by this method of applying HRP to fibres within the retina, although the success rate of these operations is low.

Previous attempts at intra-retinal labelling have not been expressly concerned to examine circumferential topography. Fawcett (1981) applied HRP to a point on the outer surface of the retina, which was left intact. It is likely that any ganglion cells labelled in this way are in a group underlying the enzyme, but retinal labelling was not examined. Fujisawa (1981; also Fujisawa et al., 1981a,b) injected HRP into the vitreous of the species he studied and made an intra-retinal cut through some of the axons. This resulted in the labelling of a contingent of fibres outside the eye, and since these are grouped together in the proximal part of their course one may presume that they originate from cells in a single area of retina. Retinal HRP was not located, however, and the axons may be from a broad sector or from an arc in peripheral retina.

Two methods of tracing fibres were adopted by Bunt (1982): axonal transport of HRP applied by a method similar to that of Fujisawa; and tracing by the morphological evidence of degeneration distal to a lesion through a cohort of axons adjacent to each other in the retina. It may be assumed that the affected ganglion cells lie peripheral to this lesion, but the only information about the position and extent of this cut is the subjective impression obtained at the time of the operation: histological evidence is lacking.

Steedman, Stirling and Gaze (1979) removed approximately three

quarters of the retina and after allowing degeneration of the corresponding axons, labelled the remainder by excising the eye and soaking the transected nerve in a solution containing cobalt ions. This is equivalent to labelling a quadrant of retina: a much broader sector than was labelled in the present experiment.

The second requirement of the experiment is that it must be possible to trace axons distally from the retina.

The histochemical method used in the present experiment (discussed on page 45) is successful in achieving morphological definition of fibres in proximal parts of the axons (plate 9) with negligible background staining; but labelling of axons in the optic tract is very faint.

The reduction in the number of visible fibres could be because some of the axons simply stop en route. This seems unlikely to be a feature of the normal pathway. The fall-off in numbers of labelled axons could also be because the retina sends axons to many other structures besides the tectum (Springer 1981); but the optic nerve has no branches, and no HRP-labelled fibres were seen to leave the tract.

A third possible explanation is that the mean concentration of HRP diminishes with increasing distance from the application site; and if the concentration of enzyme in the population of axons is normally distributed about this mean the proportion with a concentration exceeding a threshold for visibility will therefore also diminish with increasing distance. The diminished intensity of staining in the optic tract is compatible with this, but variation between fibres is not obvious.

Regenerated axons were less well stained than normal axons after comparable histochemistry. Newly regenerated axons have a smaller cross-sectional area than normal (Horder, 1974b) (and would be expected to stain less well for that reason); but 104 to 106 days after axotomy this may no longer be true.

The technical inadequacy of the present method leading to faint

labelling in the optic tract might be solved by the development of improved methods of HRP histochemistry (cf. Fujisawa, 1981; Straus, 1982; Udin and Fisher, 1983); or by increasing the amount of HRP taken up and transported by axons, perhaps by conjugating the enzyme to wheat germ agglutinin (Cook and Rankin, 1984b; Rankin and Cook, 1984), or to poly-L ornithine (Itaya, Williams and Engel, 1978) although the latter did not secure transport of HRP more than a few micrometers beyond the chiasm in a similar experiment by Fawcett (1981). It is possible that the fluorescent dye R.I.T.C. might be a suitable alternative marker in the future (page 57).

## 2) STATISTICAL ANALYSIS

A descriptive assessment of the distribution of labelled fibres is suitable in mapping those parts of the optic pathway in which relatively large numbers of fibres are distributed in an obvious pattern (e.g. the normal radial retinotopy of the goldfish optic pathway (Dawney, 1979b)). Statistical techniques are likely to be helpful only when small numbers of axons are present, in an ambiguous distribution. Steedman, Stirling and Gaze (1979) and Fujisawa (1981) omitted this: both were primarily concerned with the distribution of fibres in the dorso-ventral axis of the optic tract, and used wholemount specimens in which the distribution of fibres is not readily quantified.

Fawcett (1981) devised a formal test of orderliness, which involved standardising the shapes and areas of sections, finding the centre of the group of labelled fibres, and measuring the distance ( $r$ ) of each fibre from this. The mean of  $r^2$  ( $\overline{r^2}$ ) is used to calculate an approximation to the percentage of the cross-sectional area occupied by the labelled axons, on the grounds that when these occupy all parts of the section  $\overline{r^2}$  equals the square of the radius ( $R$ ) of the section. This is incorrect: if  $\overline{r^2} = R^2$  the axons lie around the perimeter of the sections, and therefore

have a very restricted distribution; whereas if labelled axons are uniformly distributed  $\overline{r^2}$  has a value much less than  $R^2$ .

Fawcett's index of order is higher for a disciform cluster of axons than for a linear array. The error of this is obvious if one is examining the distribution of axons originating in ganglion cells which are themselves linearly arrayed along a radius of the retina; but whatever the distribution of labelled cells, it involves an assumption about the nature of topography in the optic nerve for which there is no support.

The statistical test in the present work differs from Fawcett's work in making no direct comparison between different sections within a pathway. The most useful comparison would have been between the precision of topography in the optic tract and that evidenced by the same technique among the termination sites of the axons, in order to discover whether the former is adequate to account for the latter: but the method of labelling does not display synaptic topography. Other direct comparisons are unlikely to add much to the demonstration that the distribution of fibres may be considered random at one part of the optic pathway but not at another.

The present analysis also omits a study of serial sections. Inspection of the sections shows that the change in the precision of retinotopy is gradual, and the potential benefit of serial histology in identifying the site of an abrupt change is therefore not required.

### 3) DISTRIBUTION OF FIBRES IN THE ROSTRAL PART OF THE OPTIC NERVE

The five fish showing two separate groups of axons emerging from the eye are particularly interesting. It has previously been shown by retrograde labelling of cells following application of HRP to the dorso-medial brachium (Dawnay, 1979a; Bunt, 1982) that ventral retina sends axons through both nasal and temporal sides of the optic nerve head. In the present results it may be seen that only slightly caudal to this,

where the axons leave the eye, neighbour relationships of fibres from other parts of the retina are already breaking down. The same result has been described in the frog (Scalia and Arrango, 1983; Reh, Pitts and Constantine-Paton, 1983). This contrasts with the very precise preservation of the radial dimension of topography (Dawney, 1979b; Easter, Rusoff and Kish, 1981).

#### 4) OPTIC NERVE

The sections of the optic nerves and chiasmata demonstrate a marked reduction in circumferential topography with increasing distance from the eye. Bunt (1982) concluded that the retinotopy of the goldfish optic pathway is continuous up to the tract, and stated that the optic nerve has a structure like a ribbon folded many times in its longitudinal axis (as in related species: Scholes (1979); Ito and Murakami (1984)). It is possible that the narrow sectorial application of HRP in the present experiment labelled too few fibres to show their continuity as a tortuous line in cross-sections of this "ribbon"; or it may be that Bunt's schema of the arrangement of fibres in the rostral part of the tract is an over-interpretation: neither photographs nor drawings of his material are published, except as a diagrammatic summary. Dissociation of a cohort of fibres into two unconnected groups immediately after their exit from the eye makes it less plausible that they should really be contiguous in rostral tract.

Bunt's results concur with these in showing loss of neighbour relations: the difference is only about where and how abruptly this occurs.

This reduction of topography could occur during the growth of the axons. If so, it indicates the absence of both detailed contact guidance and selective fibre interactions as mechanisms passively and actively preserving retinotopy among the axons. But it must also be considered

whether the loss of circumferential retinotopy could have occurred subsequently, by re-modelling of the elongating optic nerve. This has been studied in Xenopus laevis, in which metamorphosis causes profound re-modelling of the optic pathway: the middle section shortens to one third of its original length, yet neither metamorphosis nor the processes of gliogenesis and myelination significantly alter the radial retinotopy (Cima and Grant, 1982). The goldfish differs from Xenopus laevis in having no metamorphosis, and in having almost all of its optic axons myelinated.

Detailed information about the radial retinotopy of the optic chiasm of the goldfish has not been published, but it is reported to be present, as in the rest of the pathway (Bunt, 1982). If it can be assumed that maturational rearrangement of axons would affect radial and circumferential dimensions of retinotopy alike, the presence of accurate radial order would suggest that circumferential orderliness had deteriorated during growth rather than after. The wide dispersion of axons from narrow sectors of retina (page 164) must consequently exclude the model proposed by Scholes (1979) for the Dempsey fish, in which it is postulated that neighbour relations are preserved within but not between quadrantic bundles. Mutual contact guidance of axons cannot be even a partial explanation for the circumferential topography of the retinotectal connections.

## 5) OPTIC TRACT

These results show that there is circumferential retinotopy in the optic tract. This is in accordance with previous results (e.g. Dawney, 1979a (goldfish); Fujisawa et al. 1981a (newt); Scalia and Fite, 1974, and Fujisawa et al., 1981b (frog)) in which larger cohorts of axons were traced. The only contradictory result is that of Steedman, Stirling and Gaze (1979), showing that in Xenopus fibres from nasal retina appear to be dispersed through the entire breadth of the optic tract.

One possible explanation for the orderliness of the optic tract is that axons may actively select their positions relative to each other or with respect to the rest of the brain. The axons in the optic nerve do not aggregate together, so the latter is more likely. Alternatively, the architecture of the optic tract may be an artefact, in that stretching of the optic pathway (during growth of the fish) might impart to the axons a distorted version of the pattern of their terminations, which are tethered on the tectum. If so, a pedantic description of the arrangement of the axons in the optic tract would be "tectotopic", rather than "retinotopic", for the circumferential dimension, just as it is "chronotopic" (being a reflection of the sequence of axon growth) in the radial dimension. The tract would probably be more accurately topographic the closer the axons are to the tectum.

The discovery that in *Xenopus* fibres from nasal retina are not obviously restricted to one or two portions of the optic tract (Steedman, Stirling and Gaze, 1979) may be construed as being consistent with the theory of axonal elongation drawing out the pattern of optic terminals into the optic tract, since these fibres terminate in caudal tectum and might be dragged across rostral areas during later growth. But this is not a necessary explanation for the finding, nor is the finding necessary for the theory to be plausible. In the goldfish axons from nasal retina are found to occupy both dorso-medial and ventro-lateral sides of the optic tract (Dawnay, 1981b). Possibly a difference in the shape of these animals causes the axons terminating caudally to be "towed" in different directions.

Differentiation between these causes of circumferential order has one significant implication about the mechanism responsible for the topography of nerve connections. If the tract is retinotopic, the axons must possess the ability to actively select their appropriate neighbours or predetermined (labelled) trajectories during their growth through the



optic tract. If the tract is tectotopic, organization of the optic terminals must take place on the tectum.

It is reported that the optic tract of Rana pipiens larvae is circumferentially retinotopic even when the optic tectum is absent, surgically removed prior to axonal growth (Reh, Pitts and Constantine-Paton, 1983). The evidence is of dorsal/ventral segregation in axons which did not terminate at the usual site of the tectum but grew caudally, many of them into the spinal cord. Detailed retinotopy was not demonstrated. Possibly the low order of topography which was shown is a reflection of the diencephalon's ability to orient the optic projection (Chung and Cooke, 1978).

Another indication of the axons' ability to select their paths is the projection of all the fibres from embryonically ventral half retinae through the dorso-medial brachium, even when their cell bodies have been transferred to a dorsal position in the retina (Straznicky, Gaze and Horder, 1979; Fawcett and Gaze, 1982). Fibres from embryonically temporal retina placed nasally have diencephalic pathways characteristic of temporal fibres, and the converse is true for nasal tissue positioned temporally (Taylor et al., 1984). But neither study helps to differentiate between tectotopic and retinotopic order in the tract (the latter dependent on positional markers laid down in early development). Combination of graft exchanges with rotation of the embryonic tectum (c.f. Chung and Cooke, 1978) would be more informative.

Holt (1983) labelled dorsal or ventral hemiretinae in *Xenopus* during embryogenesis, and found that when only about one thousand axons had formed, separate routes for these axons could already be seen. But this study does not exclude the possibility that axon paths in the optic tract are determined by the tectum, because the retinotectal projection already has an equivalent precision of retinotopy by that stage (Holt and Harris, 1983), and it is likely that this is the stage when growth, and therefore

stretching of the optic axons, is fastest.

One possible way to distinguish between the suggestion that this improved topography is an artefact of the elongation of the optic tract and the alternative theory, that there is a real reseggregation of growing axons, is to study the circumferential topography of only the youngest fibres. Because these fibres have most recently connected with the tectum they will be least affected by elongation of the tract.

This has been achieved by the labelling of a peripheral arc of retinal ganglion cells (Fawcett et al., 1984). Axons of dorsal and ventral origin were found to take specific routes to the tectum through their usual brachia, nasal through both brachia, and temporal entered the tectum directly at the rostral pole. But in this species it is not yet known how accurately circumferential retinotopy is preserved through the chiasm, so it is not certain what contribution contact guidance of axons might make to this result.

An alternative approach is to study the paths of fibres in the regenerated optic projection. Deliberate mis-routing of the tract through the path of the oculomotor nerve is followed by recovery of normal vision (Hibbard, 1967). The extent to which orderliness within this re-routed nerve was preserved is not known. Persistence of the topography of visually evoked responses behind a rostro-medial tectal lesion (Udin, 1978; Cook, Pigrim and Horder, 1983) proves that axons can form correct terminations even when the internal order of the tract, and their entry into the tectum, are abnormal.

These results do not show whether fibres are also able to select their neighbours as they grow through the optic tract. The benefit of studying regenerating tracts is that, provided the fish is not allowed to grow during the experiment, tectotopic order cannot arise by elongation of the pathway after formation of tectal synapses. The regenerated optic pathway is therefore potentially more informative than the normal.

Attardi and Sperry (1963) examined regenerating optic axons histologically, and reported that "where fibres emerge from the scar one can observe a progressive rearrangement; the fibres gather in groups becoming more and more conspicuous"; and that the axons seemed to gather in advance toward the correct brachium. (These observations and interpretations have been questioned (Horder and Martin, 1978); but the evidence from axon-tracing with HRP (against resegregation of fibres) applies strictly to the radial dimension of the projection (Dawney, 1981a,b)). Also favouring active segregation of axons is the report that following an attempt to exchange the positions of the brachia of the optic tract, axons reverted to their usual paths rather than take what appeared to be the more readily available route to the tectum (Arora and Sperry, 1962).

In contradiction to these results, Horder (1974a) found that optic fibres can grow down the wrong brachium; and Udin (1978) concluded that the paths by which regenerating axons re-enter the tectum are not significantly related to their quadrant of origin.

This question is still debated. Stuermer and Easter (1984) reported that in the normal optic tract 0.1% of axons take the "incorrect" brachium, and in the regenerated, 20%. If there were random routing one would expect the figure to be 50%. These authors reported that many fibres changed direction abruptly immediately rostral to the division of the tract, both towards and away from the appropriate side: the ratio of correct to incorrect changes was not measured.

Cook (1983) used retrograde labelling with HRP to mark axons entering the tectum at part of its rostro-medial border. Whereas in normal fish the labelled cells are in a narrow arc of rostromedial retina, after regeneration they occupy the full radius. (confirming Dawney's demonstration of failure to resegregate according to their radial coordinates); but almost all the labelled cells are in the correct

quadrant, not randomly distributed. To conclude that the axons must be segregating in the tract by selecting circumferentially appropriate neighbours (identified by unknown means), it is necessary also to demonstrate that axon distribution was initially randomized in this dimension by the optic nerve lesion.

That much is achieved by the present experiment: but rather than extrapolate to Cook's fish, it would be better to repeat his experiment adding another marker, used as here to verify complete loss of circumferential order in the same fish.

It was intended that the present investigation of the regenerated optic pathway would provide additional information by demonstrating directly whether circumferential resegregation occurs en route for the tectum. Unfortunately, in the examples studied in this experiment, staining with HRP - reaction product was inadequate for conclusions to be drawn.

In future it may also become possible to look for retinotopically selective interactions between axons growing in vitro rather than through the optic tract, to clarify the mechanism responsible for circumferential (and perhaps radial) topography among optic axon terminals.

## SUMMARY OF THIRD EXPERIMENT

Anterograde tracing of HRP - labelled axons showed that the chiasmatic part of the normal optic pathway of goldfish lacks circumferentially retinotopic order amongst its fibres. A model proposing only partial breakdown of retinotopy (Scholes, 1979) is therefore not applicable to goldfish.

The study of the dispersal of regenerated optic fibres within the optic tract excludes mutual contact guidance as a mechanism contributing to circumferential topography of terminations. Inadequacies of technique preclude conclusions about the existence of retinotopically selective re-aggregation of axons.

## CHAPTER ELEVEN

### CONCLUSIONS

Visuotectal mapping has dominated the investigation of mechanisms responsible for the generation of topography. It is uniquely able to take advantage of those attributes of the retinotectal projection which favour its use as a model in this context (page 4). As it has become increasingly evident that several factors may contribute to the development of order, there has been a corresponding increase in the need for a means of quantifying the precision of visuotectal maps, so that by controlled experiment it might be possible to discover the relative importance of each mechanism.

The principal achievement of this work is the invention of a graphical means of assessing the accuracy of retinotopy in visuotectal maps. Providing that specific, known limitations are taken into account, it is possible to extend the analysis to the derivation of a pair of numerical indices of topographic precision: the correlation coefficients in radial and circumferential dimensions. By this means it has been confirmed that regeneration can fully restore the precision of the normal projection.

The method also allows quantitative measurement of the orientation of the visuotectal map. Future application of this method will establish how variably and how reproducibly the orientation of the retinotectal projection is controlled. This may be an essential preliminary to discovering the mechanism responsible.

Portrayal of the data contained in visuotectal maps in the manner described also has the merit of demonstrating the uniformity of the retinotectal projection. The organizing process is evidently not limited to securing appropriate neighbour relationships between axon terminals, but also regulates the partition of synaptic space between them. Analysis

of conditions under which this uniformity is not maintained (perhaps low density innervation in expanded maps) will clarify the mechanism in the future.

This analysis of visuotectal maps is conducted in radial and circumferential dimensions. Indication of the overall precision is obtained by considering both together. If evaluation of bi-dimensional topography is required, it does not matter whether polar, Cartesian or any other format is used in deriving the net estimate.

Polar geometry is easiest to quantify, but Cartesian axes could be used. For example, the visual field could be divided into horizontal and vertical straight line coordinates, and the tectum similarly divided by a mediolateral and rostrocaudal grid. Alternatively, field and tectum could both be divided by lines of longitude: north and south poles lying above and below the eye for the measurement of horizontal coordinates, nasal and temporal to the eye for measurement of vertical coordinates; and similarly about the tectum. The obvious problem is that there is no independent evidence about the orientation of the map on the tectum, so the choice of tectal axes must be arbitrary, and error detrimental to the correlation between coordinates in supposedly corresponding axes. Cartesian analysis also provides no information about the orientation of the projection. If several axial directions are used, that yielding the highest correlation coefficients could be designated the "true" orientation; but the analysis is then concerned not with how accurate the pattern is, but how accurate it can be made to seem.

The relative merits of the polar geometry in these respects justify its use, even if mechanisms generating the two-dimensional order do not operate separately in radial and circumferential dimensions. But by dividing the map in this way it is potentially easy to discover whether they do.

As a general aspect of development, "pattern formation" was

originally conceived in Cartesian axes (Harrison, 1921; Wolpert, 1971). Polar format may be more appropriate to certain systems (Bryant, Bryant and French, 1977; MacDonald, 1977). Although polar terminology is commonly used to describe the arrangement of axons in the optic pathway (e.g. Scalia and Fite, 1974; Dawney, 1979; Easter, Rusoff and Kish, 1981; Cima and Grant, 1982; Fawcett et al., 1984) the array of optic terminals is more often described in Cartesian form (e.g. Sperry, 1951; Gaze, Jacobson and Szekely, 1963; Gaze, 1970; Hunt and Jacobson, 1974a; Prestige and Willshaw, 1975; Hunt and Frank, 1975; Willshaw and von der Malsburg, 1976; Hunt and Piatt, 1978). Polar geometry has been applied to the retinotectal map only where this facilitates the description of particular experiments and results (e.g. Hunt, 1977; Hunt and Ide, 1977; Tosney, Hoskins and Hunt, 1978; Ling, Ide and Hunt, 1979; Straznicky and Gaze, 1980; Conway, Feiock and Hunt, 1980; Willshaw, Fawcett and Gaze, 1983; Cooke and Gaze, 1983). Only a single visuotectal map has been called to witness the essentially polar organisation of the system: this is a map originally obtained by Hunt and Jacobson (1974b) (also printed in Hunt, 1975a) but re-interpreted by MacDonald (1976) to show radial topography in the absence of circumferential order, in the projection formed by the grafted part of a compound eye.

Map "R70", obtained after seventy days of uninterrupted regeneration following optic nerve cut, provides a striking example of accurate circumferential topography in the absence of comparable radial topography. The peripheral part of map R167 shows a similar contrast between circumferential topography and radial disorder (figures 60b,c).

These maps may be considered counterparts to MacDonald's map. It is too soon to speculate what may be responsible for these contrasted results: repetition of these examples will help to clarify the conditions under which they occur, which is the necessary preliminary to guessing at the mechanism. The implication in MacDonald's polar analysis was that



since inspection of the half-map failed to show any orderliness, it must be unlikely that the appearance of radial order would occur by chance, in the absence of a mechanism specifically responsible for it. Neither Cartesian nor polar order was quantified, and no statistical evaluation was attempted.

A rudimentary Cartesian analysis of R70 may be performed: the rank order of stimulus directions along each of three arbitrary linear axes (e.g. horizontal, vertical) is compared with the rank order of tectal recording sites along linear axes judged (subjectively) to correspond approximately to those in the visual field. In each pair one axis is rotated  $\pm 20^\circ$  and the highest Rank correlation coefficient selected. By this means, coefficients of 0.68, 0.71 and 0.88 are obtained. It is not legitimate to compare these with correlation coefficients in polar axes, because of the crudeness of their derivation. But they may be compared with other coefficients similarly obtained from two pairs of linear axes in R166a: 0.91 and 0.87. R166a is selected as the standard because (according to polar analysis) it is the most accurately topographic map. Without resorting to formal statistical comparison, it does not appear that the figures from R70 are grossly lower than those indicative of precise order. There is little justification for arguing that R70 is not retinotopic in Cartesian axes: consequently the accuracy of circumferential order does not prove that there must be an organizing mechanism operating specifically in the circumferential axis (cf. MacDonald, above). The same is true of the peripheral part of R167. That tempting conclusion must therefore be reserved; but not dismissed, for there may be other maps which show a more pronounced difference between a dimension of polar order and Cartesian chaos.

In the past literature "disorderly" visuotectal maps have occasionally been reported. Very few have been depicted in publications. One (figure 5a in Hunt and Piatt, 1978) I have analysed in polar terms

(treating the tectum as flat): radial and circumferential dimensions are equally disorderly. Nevertheless, it is only by attempting polar analysis that one can possibly show that mechanisms do organize radial and circumferential axes as separate entities, if this is so; and attempts to do so should not yet be abandoned.

Specific occasional maps, such as "A17D72" and "A20D30" make no sense if examined entirely in Cartesian terms: only with a Polar analysis can they be seen to comprise central and peripheral projections, both approximately topographic and superimposed on each other.

These experiments have been largely concerned with the possibility that the sequence of axonal arrival at the tectum might contribute to the retinotectal map. It is suggested that abnormalities of radial topography occurring after interruption of the regeneration of fibres from central retina indicate that alteration of the sequence of arrival can correspondingly alter the radial topography of their terminations.

The fact that these results are few does diminish their weight; and since other fish were treated in similar ways but yielded different results there must be substantial variation between fish which is ill-understood and difficult to control. But it is accepted that individual experimental results obtained under experimental conditions difficult to replicate precisely may yet have great significance (Feldman, Keating and Gaze, 1975). It is especially important that unusual results should be recognised as such and afforded due attention, rather than be dismissed as "un-interpretable".

Because of the radial nature of retinal growth and <sup>(perhaps)</sup> of the regeneration of the projection, invocation of time as an organizing principle obliges one to adopt radial, and therefore circumferential, axes for analysis of the mechanisms forming the map.

To insist on polar geometry would be to rebuff a long-held assumption that retino-tectal anatomy is appropriately discussed in

Cartesian terms. It is therefore necessary to weigh exactly the evidence previously interpreted as indicative of Cartesian structure.

Firstly, it was reported that if the eye primordium of Xenopus is rotated through  $180^\circ$  during a five-hour period between stages 28 and 32 of development (Nieuwkoop and Faber, 1956) its subsequent projection to the contra-lateral tectum may have a reversed antero-posterior polarity but a normal dorso-ventral axis (Jacobson, 1967, 1968). This is not a constant finding in Xenopus (Gaze, Feldman, Cooke and Chung, 1979); nor has this axial difference been found in other species (Hunt, 1975b). In predicting the results obtained by Jacobson, Sperry (1945, 1951) drew analogy with ear and limb development (Harrison, 1921). But organizational processes inside the eye need not be the same as those outside. Moreover, the orthogonal "axes" proposed by Jacobson are merely descriptive devices: reversal of one such axis is equivalent to a reversal of handedness in polar terminology (MacDonald, 1977). The same applies to other examples of "uni-axial" reversal of polarity (Hunt, 1975b).

The second argument for preferring Cartesian coordinates originates from visuo-tectal maps displaying topography in either the antero-posterior or the dorso-ventral "axis" but not both. These have been reported in two circumstances. First, visuotectal maps have been found to be random in the rostro-caudal (= antero-posterior) axis and topographic in the medio-lateral (= dorso-ventral) axis after serial transplantation of the eye bud from one side of the head to the other five times in twelve hours, beginning at stage 24 of development (Hunt, 1975a). Similarly, repeated reversal of the dorso-ventral orientation of the eye-bud may result in disorder in the medio-lateral axis with order in the rostro-caudal (Hunt, 1975a). The maps showing these results comprised few points (e.g. 11) and no attempt was made to establish whether the retinotopy within the more orderly linear axis was

significantly greater (in a statistical sense) than that of a radial or circumferential axis. It is also important to note that these maps comprised single-unit recordings, in contrast to the usual practice of recording from a group of axons together. Therefore one cannot exclude the possibility that in these fish there might be two superimposed projections each with two-dimensional topography but with opposite orientations. These might derive from neural tissue incorporated into the retina whilst the eye was in each of its two positions (cf. Holt, 1980; Gaze, Feldman, Cooke and Chung, 1979; Munro and Beazley, 1982). On the basis of this speculation one would expect that multi-unit mapping would have shown either a duplication of receptive fields on opposite sides of a vertical or horizontal meridian, or an alternation from one side to the other across such a meridian (in the event of the putative projections segregating into monotypic islands or bands: cf. Levine and Jacobson, 1975; Law and Constantine-Paton, 1981; Cook and Pilgrim, 1981). Re-examination of the experiment in this way might resolve the conflict between Hunt's conclusion and the present insistence on a polar coordinate system. Phenotypic labelling of donor retina to make it distinguishable from host tissue (e.g. by the heterozygous Oxford Nucleolar marker: Conway, Feiock and Hunt, 1980) could be used to ascertain the origin of ganglion cells in the ultimate projection.

The other report of maps topographic in the dorso-ventral field axis but not the naso-temporal is the "pattern 2" map of Gaze and Jacobson (1963) and Gaze and Keating (1970). In the former, the representation of the visual field is incomplete: two patches of field are imaged on overlapping areas of tectum; and the projection received by the rostral half tectum appears grossly topographic in the mediolateral dimension. In the latter paper, the rostral half tectum receives a more normal map, and the caudal part of the tectum receives a diffuse representation of central visual field, also showing medio-lateral but

not rostro-caudal order. In neither is it shown that the order must be interpreted as a linear axis and not a circumferential.

The other two arguments for Cartesian analysis are trivial. Translocation of an anterior sector of one right retina to the posterior pole of another right retina may be followed by the formation of a projection from the graft in which the dorso-ventral polarity is that of the host retina, not the donor (Conway, Feiock and Hunt, 1980). It has been stated that because a naso-temporal axis was retained by the graft there must be such an axis in the underlying pattern of positional values in the retina (Conway et al., op. cit.). But obviously a naso-temporal axis in an anterior or posterior sector of retina is also a radial axis: there is no reason to prefer a Cartesian format.

The last argument is based on an assessment of how much the projection from a translocated sector of retina is "skewed", and whether this corresponds to the amount expected when the graft is askew from the conventional dorso-ventral and naso-temporal axes of the eye (Conway et al., op. cit.). But the presence or absence of "skewing" within a retinotectal projection does not have a sound basis for interpretation (Straznicky, Gaze and Keating, 1974): it could indicate no more than a local distortion of the optics of the eye, or that the normally uniform curvature of the retina was disturbed by surgery. Consequently no great weight may be attached to these observations.

A final verdict on whether polar or Cartesian analysis is correct may not be possible yet. But without speculative application of the polar method now proposed, it will not be possible to identify maps showing only radial or only circumferential order; and investigation of the mechanism will be prejudiced against the polar format.

If the pattern of connections is essentially polar, this will compel a careful evaluation of certain earlier experimental results relating to the mechanisms generating topography. Combination of sectors from two

retinae in "compound eyes" (Straznicky, Gaze and Keating, 1974; Gaze and Straznicky, 1980; Straznicky and Gaze, 1980) does not affect their radial axes. The discovery that a translocated sector ( $1/4$  to  $1/3$  of the eye rudiment) forms a projection superimposed on that of the sector of the host eye corresponding to the position of origin of the donor tissue (Willshaw, Fawcett and Gaze, 1983) therefore indicates that there are retinal positional values used in the construction of a topographic projection only in respect of circumferential retinotopy. To inquire whether the same is true of radial topography one would need to construct the complementary experiment and translocate a fragment of central retina to the periphery and vice versa. If the result of this is to be interpretable, this it may also be necessary to avoid disturbance of the sequence in which axons invade the tectum.

A few experiments have contributed to the analysis of how radial topography is produced in an array of optic terminals. The optic pathway is known to be retinotopic in this dimension (Dawney, 1979b; Easter, Rusoff and Kish, 1981): contact guidance of growing axons is an adequate explanation for this. But this arrangement of axons cannot be necessary for the radial topography of optic terminals, since this is not grossly impaired by disarrangement of the optic nerve (Dawney, 1981b).

Prior to the present experiments only one other observation is specifically relevant to the generation of radial topography. This is the discovery that fibres disarranged by transection of the optic nerve do not re-segregate according to the radial coordinates of their retinal origins en route for the tectum (Dawney, 1981a). If recognition of positional values does contribute to topography in this dimension it must occur only on the tectum.

It is most important that comparable studies should be performed on the circumferential dimension, as intended in chapters 9 and 10: experiments showing that regenerating axons re-enter the tectum not

randomly but by routes dependent on the circumferential coordinates of their retinal origin (Cook, 1983; Stuermer and Easter, 1984), should be repeated with a guarantee that all circumferential topography in the optic pathway was abolished by the lesion. If this is achieved, the contrast between radial and circumferential dimensions will be further evidence of the need for polar analysis to investigate polar mechanisms.

Continuing ignorance of the mechanisms generating radial topography accentuates the need to inquire whether temporal sequence makes any contribution. The technical limitations of the present experiments have been discussed in detail in chapters 4 and 8. The salient points are these. (i) The method used in chapter 3 to mark optic fibres does not allow an estimation of the accuracy of retinotopy in the observed sequence of retinal labelling. (ii) The sequence in which optic fibres re-innervated the tectum was not demonstrated in the fish whose optic nerve regeneration was partly interrupted. (iii) It could not be shown that the visually evoked potentials indicating mis-placed projections from central retina to peripheral tectum originated in stable synaptic terminals. These are all important points, and ways in which future experiments might be improved in these respects have been considered.

Despite these limitations the results are of great interest. The fact that gross errors of radial topography may be induced merely by interrupting the regeneration of axons from central retina shows that the sequence in which optic fibres re-innervate the tectum can influence the radial coordinate of at least their initial connection with the tectum. No other explanation is obviously adequate: partial transection of the optic nerve cannot be expected to disrupt locus-specific ganglion cell labels, nor to affect the tectum at all. The fact that some axons are cut twice is not likely to cause disorganisation: repeated transection (by crushing) of the whole population of axons has been found to have no effect upon the retinotopy of subsequent connections (Horder, 1974a). Nor

may the effect of the lesion be attributed to alteration of the relative positions of the axons in the nerve. Axons regenerating through the optic tract with no radial retinotopy form an orderly map of connections sooner after axotomy (e.g. 28 days: Dawney, 1981b) than the present fibres formed erroneous projections.

The distortions of radial topography observed in some of these fish do not prove that the sequence of axonal arrival is sufficient by itself to confer detailed radial retinotopy on the projection: other models must still be considered, both individually and in conjunction with sequential arrival of axons.

Six maps (A17D:42,81; A20D:30,31; A33D:61, 146) show that central retina may project simultaneously to both central and peripheral tectum: this might be construed as indicating that there is also some mechanism by which axons actively select radially appropriate pre-synaptic neighbours. If this alone were sufficient to determine the final positions of optic terminals one would expect that in this group of results increasing duration of regeneration would be marked by a progressive change from anomalous to correct patterns of termination. This is not seen over the time scale normally associated with the formation of a radially retinotopic map. But because there is clearly some variation between fish the only conclusive way to resolve this uncertainty would be to re-map individual fish.

One solitary result is highly suggestive of active selection of termination sites within the radial dimension of the fibre array. A miniature eye (its growth arrested by treatment with fluorodeoxyuridine and containing about 4000 ganglion cells) formed a radially expanded projection on the contralateral tectum until a projection from a normal eye was added: the first projection shrank down to occupy only the small area of tectum appropriate to ganglion cells close to the optic nerve head (Hunt, 1977). This indicates that interactions between fibres



actively control the radial coordinates of their termination sites (or both coordinates, if Cartesian axes are used). This interesting result needs confirmation.

If there is some mechanism actively generating radial topography the contribution, in regeneration, of a radially specific sequence of arrival might be the prevention of gross errors of topography and the avoidance of delay in the recovery of pattern. But the contribution to development of an active mechanism operating in the radial dimension is not obvious (see page 32). Teleological speculation raises the possibility that the phenomenon of "sliding" of retino-tectal connections during growth (Gaze, Chung and Keating, 1972; Chung, Keating and Bliss, 1974; Gaze, Keating and Chung, 1974; Freeman, 1977; Easter and Stuermer, 1984) might require active preservation of neighbour relations in both circumferential and radial dimensions against a disruptive effect of movement; but evidence for this is lacking.

Another way in which sequential arrival of axons might play a contributory role may be considered in relation to the theoretical model of Willshaw and von der Malsburg (1976). This model was originally proposed to account for a two-dimensional map: it is equally applicable to just the radial dimension. It proposes that optic terminals continually exchange positions, except when juxtaposed axons happen to come from neighbouring ganglion cells: in this event they form more durable connections with the subjacent tectum. (The information imagined to be carried by optic axons allows discrimination between neighbours and non-neighbours: it does not indicate the exact origin of each fibre. Consequently it does not explain the result of Hunt (1977) recounted above). Topography would gradually evolve; but if the arborisation of each axon does not spread initially over the entire tectal surface (which would have enabled it to identify all of its correct neighbours simultaneously), it is necessary to stipulate that organisation must

begin at a single site and spread from that. Without this proviso, the evolution of the map may become trapped in local optima. For example, in the absence of this condition a row of ganglion cells ABCDEF could form two partial maps DEFABC which cannot be rearranged without increasing disorder. This might not occur unless the topography is inherently unstable; which would negate any organising mechanism. During development order does occur initially in a single part of the map and does spread from there, because of the radial sequence of axonal arrival (Johns, 1977; Meyer, 1978). Since a similar sequence now appears to occur in regeneration one must allow the possibility that there may be synergism between these "active" and "passive" models.

The contribution of sequential arrival of axons to the organisation of the retino-tectal projection of the goldfish will remain uncertain until technical improvements permit an assessment of the accuracy of retinotopy in this sequence (further defining the capacity of this "passive" model to generate accurate topography); and until there is further examination of the axons' power to select radially appropriate neighbours (which is the basis of the "active" model).

A central - to - peripheral sequence of optic re-innervation is not confined to the goldfish tectum: regenerated central retina is older than regenerated peripheral retina in the newt (Gaze and Watson, 1968), and there is a centrifugal sequence in the newt's retinotectal regeneration (Cronly-Dillon, 1968).

It would be gratifying to prove that the retinotectal projection can legitimately be considered a "model" typifying developmental mechanisms of general applicability. This validation will be possible only post hoc; but it is interesting to consider whether sequential arrival of axons can contribute to topography in other neural systems.

To date, no other neural projection been shown to depend upon time for the generation of spatial order. There is a radial pattern of growth

in the chick retina (Kahn, 1974; Rager, 1980b), but although it has been suggested that this might contribute to radial topography of retinotectal connections, this has not been tested by experiment. The retinae of albino rats (Braekevelt and Hollenberg, 1970) and mice (Sidman, 1960), and possibly also cats (Rapaport and Stone, 1982) also grow by peripheral increments.

There is a retinotopic sequence in the formation of retino-laminar connections in the locust (Anderson, 1978); and a correlation between cell birth date and synaptic connectivity in the visual projection of Daphnia (Flaster and Macagno, 1980). In each case extirpation of part of the retina is followed by abnormal patterns of connection interpreted as excluding rigidly specific pairing of pre- and post-synaptic cells (Anderson, 1978; Macagno, 1978). But it is not yet possible to infer that sequential synaptogenesis, rather than passive contact guidance of axons or actively selective interactions between optic fibres is responsible for the topography (Lopresti, Macagno and Levinthal, 1973).

One recent investigation has shown that the sequence in which the earliest optic axons leave the *Xenopus* eye (dorsal before ventral: Holt and Harris, 1983) may be altered without disturbance to the polarity of the mature projection (Holt, 1984). In view of the evidence already discussed that the orientation of the projection is "actively" controlled in some way, this result is not surprising; and it has no bearing on the contribution of sequence to other aspects of pattern.

The significance of time has also been considered in respect of one non-visual system. In mammals there are extrinsic, association and commissural afferents to the hippocampus and dentate gyrus: each terminates upon a restricted portion of the surfaces of pyramidal and granule cells. In the rat dentate gyrus it has been found that cells form in a well-defined temporal sequence; and that the ratio of ipsi- and contra-lateral inputs varies systematically along the axis of generation

of cells (Gottlieb and Cowan, 1972). Since the number of synaptic sites is uniform, these authors propose that there must be competition for a limited number of sites; and that it is the time of an axon's arrival which determines where it forms connections.

It is intended that the work presented here should provoke further inquiries into diverse aspects of retinotectal pattern formation: the end of this thesis is certainly far from being a natural conclusion to the investigations it describes. In these pages I have argued (from previous workers' contributions) that differential affinities between regions of the tectum and axons from regions of the retina appear to be able to regulate the orientation of the set of optic terminals; and that the topographic pattern in which these terminals are deployed is generated by at least two processes. These are the active recognition by axons of appropriate neighbours, and refinement of crude topography by interactions dependent on visual function. Nor can one exclude the possibility that contact guidance of axons also contributes by increasing the number of axons which form correctly placed connections. To these it now appears necessary to add the temporal sequence in which axons arrive at their target; although it is still not clear exactly what role this plays.

While reviews of this subject tend to agree on the need for a "multi-factorial" explanation for the pattern of retinotectal connections (Cook and Horder, 1977; Fraser and Hunt, 1980; Constantine-Paton 1982; Meyer, 1982b) there has been little agreement about how the work of organisation is to be divided. In the present work it is suggested that, rather than grapple with two-dimensional topography as a whole, future studies should consider radial and circumferential dimensions of topography separately, if they are to define the contribution made by

each mechanism. Quantification of topography may be a valuable, perhaps essential, contribution towards the same end. The success of the simple model for polar analysis of visuotectal maps used in this thesis encourages the hope that, with improvement in experimental technique, an extension of this work will greatly clarify the process of development.

## BIBLIOGRAPHY

- Adams, J.C. (1977) Technical considerations on the use of horseradish peroxidase as a neuronal marker. Neuroscience 2 141 - 145
- Adams, J.C. (1981) Heavy metal intensification of DAB-based HRP reaction product. J. Histochem. Cytochem. 29 775
- Anderson, H. (1978) Postembryonic development of the visual system of the locust, Schistocerca gregaria. II. An experimental investigation of the formation of the retino-lamina projection. J. Embryol. exp. Morph. 46 147 - 170
- Apter, J.T. (1945) Projection of the retina on superior colliculus of cats. J. Neurophysiol. 8 123 - 134
- Arnett, D.W. (1978) Statistical dependence between neighbouring retinal ganglion cells in goldfish. Exp. Brain Res. 32 49 - 53
- Arnett, D.W. and Spraker, T.E. (1981) Cross correlation of the maintained discharge of retinal ganglion cells. J. Physiol. 317 29 - 47
- Arora, H. L. and Grinnell, A. D. (1976) Recovery of visual function in the "compressed" hemitectum of goldfish. Anat. Rec. 184 574 - 575
- Arora, H.L. and Sperry, R.W. (1962) Optic nerve regeneration after surgical cross-union of medial and lateral optic tracts. Amer. Zool. 2 389
- Arora, H.L. and Sperry, R.W. (1963) Colour discrimination after optic nerve regeneration in the fish Astronotus ocellatus. Dev. Biol. 7 234 - 243
- Attardi, D.G. and Sperry, R.W. (1963) Preferred selection of central pathways by regenerating optic fibres. Exp. Neurol. 7 46 - 64
- Bak, T.A. and Lichtenberg, J. (1966) Mathematics for scientists. Benjamin, N.Y.
- Barbera, A.J. (1975) Adhesive recognition between developing retinal cells and optic tecta of the chick embryo. Dev. Biol. 46 167 - 191

- Barbera, A.J., Marchase, R.B. and Roth, S. (1973) Adhesive recognition and retinotectal specificity. Proc. Natl. Acad. Sci. U.S.A. 70 2482 - 2486
- Berman, N. and Hunt, R.K. (1975) Visual projections to the optic tecta in *Xenopus* after partial extirpation of the embryonic eye. J. comp. Neurol. 162 23 - 42
- Biber, M.P., Kneisley, L.W. and LaVail, J.M. (1978) Cortical neurons projecting to cervical and lumbar enlargements on the spinal cord of young and adult Rhesus monkeys. Exp. Neurol. 59, 492 - 508
- Bodick, N. and Levinthal, C. (1980) Growing optic nerve fibres follow neighbours during embryogenesis. Proc. natl. Acad. Sci. U.S.A. 77 4374 - 4378
- Bohn, R.C. and Reier, P.J. (1982) Anomalous axonal outgrowth at the retina caused by injury to the optic nerve or tectal ablation in adult *Xenopus*. J. Neurocytol. 11 211 - 234
- Bonhoeffer, F. and Huff, J. (1982) in vitro experiments of axon guidance demonstrating an antero-posterior gradient on the tectum. EMBO J. 1 42 - 45
- Braekevelt, C.R. and Hollenberg, M.J. (1970) The development of the retina in the albino rat. Am. J. Anat. 127 281 - 302
- Bryant, P.J. Bryant, S.V. and French, V. (1977) Regeneration and pattern formation. Sci. Am. 237 66-81.
- Bunge, M.B. (1977) Initial endocytosis of peroxidase or ferritin by growth cones of cultured nerve cells. J. Neurocytol. 6 407 - 439
- Bunt, S.M. (1982) Retinotopic and temporal organization of the optic nerve and tracts in the adult goldfish. J. comp. Neurol. 206 209-226

- Bunt, S.M., Horder, T.J. and Martin, K.A.C. (1978) Evidence that optic fibres regenerating across goldfish optic tectum may be assigned termination sites on a "first-come, first-served" basis. J. Physiol. 276 45 - 46 P
- Bunt, S.M., Horder, T.J. and Martin, K.A.C. (1979) The nature of the nerve fibre guidance mechanism responsible for an orderly central visual projection. pp.331 - 343 in: "Developmental Biology of Vision", ed. R.D. Freeman. Plenum, N. Y.
- Burgen, A.S.V. and Grafstein, B. (1962) Retinotectal connections after retinal regeneration. Nature 196 898 - 899
- Chung, S.H. (1974) In search of the rules for nerve connections. Cell 3 201 - 205
- Chung, S.H. and Cooke, J. (1978) Observations on the formation of the brain and of nerve connections following embryonic manipulation of the amphibian neural tube. Proc. roy. Soc. Lond. B 201 335 - 373
- Chung, S.H., Gaze, R.M. and Stirling, R.V. (1973) Abnormal visual function in *Xenopus* following stroboscopic illumination. Nature New Biol. 246 186 - 188
- Chung, S.H., Keating, M.J. and Bliss, T.V.P. (1974) Functional synaptic relations during the development of the retinotectal projection in amphibians. Proc. roy. Soc. Lond. B 187 449 - 459
- Cima, C. and Grant, P. (1982) Development of the optic nerve in *Xenopus laevis*. II. Gliogenesis, myelination and metamorphic remodelling. J. Embryol. exp. Morph. 72 251 - 267
- Constantine-Paton, M. (1982) The retinotectal hookup: the process of neural mapping. pp. 317 - 349 in: "Developmental order: its origin and regulation". Ed. S. Subtelny. Alan R. Liss, N. Y.
- Constantine-Paton, M. and Law, M.I. (1978) Eye-specific termination bands in tecta of three-eyed frogs. Science 202 639 - 641



- Conway, K., Feiock, K. and Hunt, R.K. (1980) Polyclones and pattern in growing *Xenopus*. Curr. topics dev. Biol. 15 217 - 319
- Cook, J.E. (1979) Interactions between optic fibres controlling the locations of their terminals in goldfish optic tectum. J. Embryol. exp. Morph. 52 89-103
- Cook, J.E. (1983) Tectal paths of regenerated optic axons in the goldfish: evidence from retrograde labelling with horseradish peroxidase. Exp. Brain Res. 51 433 - 442
- Cook, J.E. and Horder, T.J. (1974) Interactions between optic fibres in their regeneration to specific sites in the goldfish tectum. J. Physiol. 241 89 - 90P
- Cook, J.E. and Horder, T.J. (1977) The multiple factors determining retinotopic order in the growth of optic fibres into the tectum. Phil. Trans. roy. Soc. B 278 261 - 276
- Cook, J.E. and Pilgrim, A.J. (1981) Reproducible terminal segregation in doubly-innervated goldfish tecta : electrophysiological and autoradiographic assessment. J. Physiol. 325 10P
- Cook, J.E., Pilgrim, A.J. and Horder, T.J. (1983) Consequences of misrouting goldfish optic axons. Exp. Neurol. 79 830-844
- Cook, J.E. and Rankin, E.C.C. (1984) Use of a lectin peroxidase conjugate (WGA - HRP) to assess the retinotopic precision of goldfish optic terminals. Neurosci. L. 48 61 - 66
- Cook, J.E., Rankin, E.C.C. and Stevens, H.P. (1983) A pattern of optic axons in the normal goldfish tectum consistent with the caudal migration of optic terminals during development. Exp. Brain Res. 52 147 - 151
- Cooke, J. and Gaze, R.M. (1983) The positional coding system in the early eye rudiment of *Xenopus laevis*, and its modification after grafting operations. J. Embryol. exp. Morph. 77 53 - 71
- Cooper, S., Daniel, P.M. and Whitteridge, D. (1953) Nerve impulses in the brain stem and cortex of the goat. J. Physiol. 120 514 - 527

- Cragg, B.L. (1970) What is the signal for chromatolysis? Brain Res. 23  
1 - 21
- Cronly-Dillon, J.R. (1968) Pattern of retinotectal connections after retinal regeneration. J. Neurophysiol. 31 410 - 418
- Cronly-Dillon, J.R. and Glaizner, B. (1974) Specificity of regenerating optic fibres for left and right optic tecta in goldfish. Nature 257  
505 - 507
- Cunningham, T.J. and Speas, G. (1975) Inversion of anomalous uncrossed projections along the mediolateral axis of the superior colliculus. Implications for retino-collicular specificity. Brain Res. 88 73 - 79
- Dawney, N.A.H. (1979a) Retinotopic architecture in goldfish optic pathway. J. Physiol. 289 50 - 51P
- Dawney, N.A.H. (1979b) "Chronotopic" organisation of goldfish optic pathway. J. Physiol. 296 13 - 14P
- Dawney, N.A.H. (1981a) Fibre ordering within regenerated optic pathways of goldfish. J. Physiol. 317 76 - 77P
- Dawney, N.A.H. (1981b) "Studies in central nervous regeneration and the topography of nerve fibre growth." D.Phil. thesis, Oxford University.
- Dawney, N.A.H. and Glees, F.P. (1981) Mapping the primate corticospinal pathway. J. Anat. 133 124
- Draper, N.R. and Smith, H. (1960) Applied regression analysis. 2nd Ed. Wiley, N.Y.
- Drury, R.A.B. and Wallington, E.A. (1967) Carleton's Histological Technique. 4th ed. O.U.P.
- Easter, S.S., Rusoff, A.C. and Kish, P.E. (1981) The growth and organisation of the optic nerve and tract in juvenile and adult goldfish. Neuroscience 1 793 - 811

- Easter, S.S. and Stuermer, C.A.O. (1984) An evaluation of the hypothesis of shifting terminals in goldfish optic axons. J. Neuroscience 4 1052 - 1063
- Fawcett, J.W. (1981) How axons grow down the *Xenopus* optic nerve. J. Embryol. exp. Morph. 65 217 - 233
- Fawcett, J.W. and Gaze, R.M. (1982) The retinotectal fibre pathways from normal and compound eyes in *Xenopus*. J. Embryol. exp. Morph. 72 19 - 37
- Fawcett, J.W., Taylor, J.S.H., Gaze, R.M., Grant, P. and Hirst, E. (1984) Fibre order in the normal *Xenopus* optic tract, near the chiasma. J. Embryol. exp. Morph. 83 1-14.
- Fawcett, J.W. and Willshaw, D.J. (1982) Compound eyes project stripes on the optic tectum in *Xenopus*. Nature 296 350 - 352
- Feldman, J.D. and Gaze, R.M. (Appendix by Neil MacDonald) (1975) The development of half eyes in *Xenopus* tadpoles. J. comp. Neurol. 162 13 - 22
- Feldman, J.D., Keating, M.J. and Gaze, R.M. (1975) Retinotectal mismatch: a serendipitous experimental result. Nature 253 445 - 446
- Flaster, E. and Macagno, E. (1980) Correlation between cell birth date and synaptic connectivity in a neuronal array. Soc. for Neuroscience, Abstracts vol 6 139.1
- Fraser, S.E. (1980) A differential adhesion approach to the patterning of nerve connections. Dev. Biol. 79 453 - 464
- Fraser, S.E. and Hunt, R.K. (1980a) Retinotectal plasticity in *Xenopus*: anomalous projection following late larval eye removal. Dev. Biol. 79 444 - 452
- Fraser, S.E. and Hunt, R.K. (1980b) Retinotectal specificity - models and experiments in search of a mapping function. Ann. Rev. Neurosci. 3 319 - 352

- Freeman, J.A. (1977) Possible regulatory function of acetyl choline receptor in maintainance of retinotectal synapses. Nature 269 218 - 222
- Fujisawa, H. (1981) Retinotopic analysis of fibre pathways in the regenerating retinotectal system of the adult newt Cynops pyrrhogaster. Brain Res. 206 27 - 37
- Fujisawa, H., Thanos, S. and Schwarz, U. (1984) Mechanisms in the development of retinotectal projections in the chick embryo studied by surgical deflection of the surgical pathway. Dev. Biol. 102 356 - 367
- Fujisawa, H., Wanatabe, K., Tani, N. and Ibata, Y. (1981a) Retinotopic analysis of fibre pathways in amphibians. I. The adult newt Cynops pyrrhogaster. Brain Res. 206 9 - 20
- Fujisawa, H., Wanatabe, K., Tani, N. and Ibata, Y. (1981b) Retinotopic analysis of fibre pathways in amphibians. II. The frog Rana nigromaculata. Brain Res. 206 21 - 26
- Gaze, R.M. (1958) The representation of the retina on the optic lobe of the frog. Quart. J. exp. Physiol. 43 209 - 214
- Gaze, R.M. (1959) Regeneration of the optic nerve in Xenopus laevis. Quart. J. exp. Physiol. 44 290 - 308
- Gaze, R.M. (1960) Regeneration of the optic nerve in amphibia. Intl. Rev. Neurobiol. 2 1 - 40
- Gaze, R.M. (1970) "The formation of nerve connections." Academic Press, N. Y.
- Gaze, R.M. and Fawcett, J.W. (1983) Pathways of Xenopus optic fibres regenerating from normal and compound eyes under various conditions. J. Embryol. exp. Morph. 73 17 - 38
- Gaze, R.M. and Grant, P. (1978) The diencephalic course of regenerating retinotectal fibres in Xenopus tadpoles. J. Embryol. exp. Morph. 44 201 - 216

*not alphabetical*

- Gaze, R.M., Feldman, J.D., Cooke, J. and Chung, S.H. (1979) The orientation of the visuotectal map in *Xenopus*: developmental aspects. J. Embryol. exp. Morph. 53 39 - 66
- Gaze, R.M. and Jacobson, M. (1963) A study of the retinotectal projection during regeneration of the optic nerve in the frog. Proc. Roy. Soc. Lond. B 157 420-448
- Gaze, R.M., Jacobson, M. and Szekely, G. (1963) The retinotectal projection in *Xenopus* with compound eyes. J. Physiol. 165 484-499
- Gaze, R.M. and Keating, M.J. (1970) Further studies on the restoration of the contralateral retinotectal projection following regeneration of the optic nerve in the frog. Brain Res. 21 183-195
- Gaze, R.M., Keating, M.J. and Chung, S.H. (1974) The evolution of the retinotectal map during development in *Xenopus*. Proc. roy. Soc. Lond. B 185 301 - 330
- Gaze, R.M., Keating, M.J., Ostberg, A. and Chung, S.H. (1979) The relationship between retinal and tectal growth in larval *Xenopus*: implications for the development of the retinotectal projection. J. Embryol. exp. Morph. 53 103 - 143
- Gaze, R.M. and Sharma, S.C. (1970) Axial differences in the re-innervation of the goldfish optic tectum by regenerating optic nerve fibres. Exp. Brain Res. 10 171 - 181
- Gaze, R.M. and Straznicky, C (1980) Stable programming for map orientation in disarranged embryonic eyes in *Xenopus*. J. Embryol. exp. Morph. 55 143 - 165
- Gaze, R.M. and Watson, W.E. (1968) Cell division and migration in the brain after optic nerve lesion. pp. 53 - 67 in: G.E.W. Wolstenholme and M. O'Connor (eds.) "Growth of the nervous system". Ciba Symposium. Churchill.

- George, S.A. and Marks, W.B. (1974) Optic nerve terminal arborizations in the frog; shape and inferred from electrophysiological measurements. Exp. Neurol. 42 467 - 482
- Goldberg, S. (1974) Studies on the mechanisms of development of the visual pathways in the chick embryo. Dev. Biol. 36 24 - 43
- Gottlieb, D.I. and Cowan, W.M. (1972) Evidence for a temporal factor in the occupation of available synaptic sites during the development of the dentate gyrus. Brain Res. 41 452 - 456
- Gottlieb, D.I. and Glaser, L.A. (1980) Cellular recognition during neural development. Ann. Rev. Neurosci. 3 303 - 318
- Griffini, L. and Marcho, G. (1889) Sur la regeneration totale de la retine chez les tritons. Arch. Ital. Biol. 12 82-89
- Grover, B.G. and Sharma, S.C. (1981) Organisation of extrinsic tectal connections in goldfish. J. comp. Neurol. 196 471 - 488
- Halfter, W., Claviez, M. and Schwarz, U. (1981) Preferential adhesion of tectal membranes to anterior embryonic chick retinal neurites. Nature, 292 67 - 70
- Halfter, W. and Deiss, S. (1984) Axon growth in embryonic chick and quail retinal whole mounts in vitro. Dev. Biol. 102 344 - 355
- Harris, W.A. (1980) The effects of eliminating impulse activity on the development of the retinotectal projection in salamanders. J. comp. Neurol. 194 303 - 317
- Harris, W.A. (1984) Axonal pathfinding in the absence of normal pathways and impulse activity. J. Neurosci. 4 1153 - 1162
- Harrison, R.G. (1910) The outgrowth of the nerve fibre as a mode of protoplasmic movement. J. exp. Zool. 9 787 - 846
- Harrison, R.G. (1921) On relations of symmetry in transplanted limbs. J. exp. Zool. 32 1 - 136

- Hibbard, E. (1967) Visual recovery following regeneration of the optic nerve through the oculomotor nerve root in *Xenopus*. Exp. Neurol. 19 350 - 356
- Hoffman, P.N. and Lasek, R.J. (1980) Axonal transport of the cytoskeleton in regenerating motor neurones : constancy and change. Brain Res. 202 317-333
- Holt, C.E. (1980) Cell movements in *Xenopus* eye development. Nature 287 850 - 852
- Holt, C.E. (1983) Topography of the initial retinotectal projection. Prog. Brain Res. 58 339-345
- Holt, C.E. (1984) Does timing of axon outgrowth influence initial retinotectal topography in *Xenopus*? J. Neurosci. 4 1130 - 1152
- Holt, C.E. and Harris, W.A. (1983) Order in the initial retinotectal map in *Xenopus*: a new technique for labelling growing nerve fibres. Nature 301 150 - 152
- Hope, B.A., Hammond, B.J. and Gaze, R.M. (1976) The "arrow" model; retinotectal plasticity and map formation in the goldfish visual system. Proc. roy. Soc. Lond. B 194 447 - 466
- Horder, T.J. (1971a) Retention, by fish optic nerve fibres regenerating to new terminal sites on the tectum, of "chemospecific" affinity for their original sites. J. Physiol. 216 53 - 55P
- Horder, T.J. (1971b) The course of recovery of the retinotectal projection during regeneration of the fish optic nerve. J. Physiol. 217 53-54 P
- Horder, T.J. (1974a) Changes of fibre pathways in the goldfish optic tract following regeneration. Brain Res. 72 41 - 52
- Horder, T.J. (1974b) Electron microscopic evidence in goldfish that different optic nerve fibres regenerate selectively through specific routes into the tectum. J. Physiol. 241 84 - 85P

- Horder, T.J. and Martin, K.A.C. (1977) Translocation of optic fibres in the tectum may be determined by their stability relative to surrounding fibre terminals. J. Physiol. 271 23 - 24P
- Horder, T.J. and Martin, K.A.C. (1978) Morphogenetics as an alternative to chemospecificity in the formation of nerve connections. In: "Cell - cell recognition", S.E.B. Symposium XXXII Ed. A.S.G. Curtis. C.U.P.
- Hunt, R.K. (1975a) The cell cycle, cell lineage and neuronal specificity. pp. 43 - 62 in: Cell cycle and cell differentiation. (Results and Problems in Cell Differentiation, vol. 7) eds. J. Reinart and H. Holtzer. Springer, Berlin.
- Res. Probs. Cell Differentiation 7 43 - 62.
- Hunt, R.K. (1975b) Position - dependent differentiation of neurons. In: ICN - UCLA Symposia on molecular and cellular biology. Vol. 2: Developmental biology. Eds. D. McMahon and C.F. Fox. Pub.: W.A. Benjamin, Menlo Park.
- Hunt, R.K. (1976) Cell lineage and cell interactions in neuronal differentiation. p. 11 In : Progress in Differentiation Research. Ed. N. Muller- Berat Pub.: North-Holland, Amsterdam
- Hunt, R.K. (1977) Competitive retinotectal mapping in *Xenopus*. Biophys. J. 17 128a
- Hunt, R.K. and Berman N. (1975) Patterning of neuronal locus specificities in retinal ganglion cells after partial extirpation of the embryonic eye. J. comp. Neurol. 162 43 - 70
- Hunt, R.K. and Frank, E.D. (1975) Neuronal locus specificity: transrepolarisation of *Xenopus* embryonic retina after the time of axial specification. Science 189 563 - 565
- Hunt, R.K. and Ide, C.F. (1977) Radial propagation of positional signals for retinotectal patterns in *Xenopus*. Biol. Bulletin 153 430-443 .



- Hunt, R.K. and Jacobson, M. (1972) Specification of positional information in retinal ganglion cells of *Xenopus*: assays for analysis of the unspecified state. Proc. natl. Acad. Sci. U.S.A. 69 780 - 783
- Hunt, R.K. and Jacobson, M. (1973) Neuronal locus specificity: altered pattern of spatial development in fused fragments of embryonic *Xenopus* eyes. Science 180 509 - 511
- Hunt, R.K. and Jacobson, M. (1974a) Neuronal specificity revisited. Curr. Top. dev. Biol. 8 203 - 259
- Hunt, R.K. and Jacobson, M. (1974b) Development of neuronal locus specificity in *Xenopus* retinal ganglion cells after surgical eye transection or after fusion of whole eyes. Dev. Biol. 40 1 - 15
- Hunt, R.K. and Piatt, J. (1978) Cross species axial signalling, with re-alignment of retinal axes, in embryonic *Xenopus* eyes. Dev. Biol. 62 44 - 51
- Ide, C.F. and Hunt, R.K. (1978) Positional signalling in chimeric *Xenopus* retinae. Biophys. J. 21 110a
- Ide, C.F., Kosofsky, B.E. and Hunt, R.K. (1979) Control of pattern duplication in the retinotectal system of *Xenopus*: suppression of duplication by eye fragment interactions. Dev. Biol. 69 337 - 360
- Itaya, S.K., Williams, T.H. and Engel, E.L. (1978) Anterograde transport of horseradish peroxidase enhanced by poly-L-ornithine. Brain Res. 150 170 - 176
- Ito, H. and Murakami, T. (1984) Retinal ganglion cells in two teleost species, Sebasticus marmoratus an Navodon modestus. J. comp. Neurol. 229 80 - 96
- Jacobson, M. (1967) Retinal ganglion cells: specification of central connections in larval *Xenopus laevis*. Science 155 1106 - 1108
- Jacobson, M. (1968) Development of neuronal specificity in retinal ganglion cells of *Xenopus*. Dev. Biol. 17 202 - 218

- Jacobson, M. and Gaze, R.M. (1964) Types of visual response from single units in the optic tectum and optic nerve of the goldfish. Quart. J. exp. Physiol. 49 199 - 209
- Jacobson, M. and Gaze, R.M. (1965) Selection of appropriate tectal connections by regenerating optic nerve fibres in adult goldfish. Exp. Neurol. 13 418 - 430
- Jacobson, M. and Levine, R.L. (1975) Plasticity in the adult frog brain: filling the visual scotoma after excision or translocation of parts of the optic tectum. Brain Res. 88 309 - 345
- Johns, P.R. (1977) Growth of the adult goldfish eye. III. Source of the new retinal cells. J. comp. Neurol. 176 343 - 358
- Johns, P.R. and Easter, S.S. (1977) Growth of the adult goldfish eye. II. Increase in retinal cell number. J. comp. Neurol. 176 331 - 342
- Kahn, A.J. (1974) An autoradiographic analysis of the time of appearance of neurons in the developing chick retina. Dev. Biol. 38 30 - 40
- Kristensson, K. and Olsson, Y. (1976) Retrograde transport of horseradish peroxidase in transected axons. III. Entry into axons and subsequent localization in perikaryon. Brain Res. 115 201 - 213
- Lanners, H.N. and Grafstein, B. (1980) Early stages of axonal regeneration in the goldfish optic tract: an electron microscopic study. J. Neurophysiol. 9 733 - 751
- LaVail, J.H. (1975) Retrograde cell degeneration and retrograde transport techniques. pp. 217 - 248 in: "The use of axonal transport for studies of neuronal connectivity". Eds. W.M. Cowan and M. Cuenod. Pub.: Elsevier.
- LaVail, J.H. and LaVail, M.M. (1974) The retrograde intra-axonal transport of horseradish peroxidase in the chick visual system: a light and electron microscopic study. J. comp. Neurol. 157 303 - 358

- Law, M.I. and Constantine-Paton, M. (1981) Anatomy and physiology of experimentally-produced striped tecta. J. Neurosci. 1 741 - 759
- Lazar, G. (1971) The projection of the retinal quadrants on the optic centres in the frog. A terminal degeneration study. Acta morph. Acad. Sci. Hung. 19 325 - 334
- Levine, R.L. and Jacobson, M. (1974) Deployment of optic nerve fibres is determined by positional markers in the frog's tectum. Exp. Neurol. 43 527 - 538
- Levine, R.L. and Jacobson, M. (1975) Discontinuous mapping of retina on to tectum innervated by both eyes. Brain Res. 98 172 - 176
- Lieberman, A.R. (1974) Some factors affecting retrograde neuronal responses to axonal lesions. pp. 71 - 105 in: Essays on the nervous system (Festschrift for J.Z.Young) Fds. R. Bellairs and E.G. Grey. Clarendon, Oxford
- Ling, R.T.K., Ide, C.F. and Hunt, R.K. (1979) Control of pattern duplication in the retinotectal system of *Xenopus*: induction of duplication in eye fragments by secondary cuts. Dev. Biol. 69 361 - 374
- Lopresti, V., Macagno, E.R. and Levinthal, C. (1973) Structure and development of neuronal connections in isogenic organisms: cellular interactions in the development of the optic lamina in *Daphnia*. Proc. natl. Acad. Sci. 70 433 - 437
- Macagno, E.R. (1978) A mechanism for the formation of synaptic connections in the arthropod visual system. Nature 275 318 - 320
- Macagno, E.R. (1981) Cellular interactions and pattern formation in the development of the visual system of *Daphnia magna* (Crustacea, Branchiopoda). II. Induced retardation of optic nerve ingrowth results in a delay in a delay in laminar neuron differentiation. J. Neurosci. 1 945 - 955

- MacDonald, N. (1975) Appendix to: "The development of half-eyes in *Xenopus* tadpoles" by Feldman, J.D. and Gaze, R.M. J. comp. Neurol. 162 13 - 22
- MacDonald, N. (1977) A polar coordinate system for positional information in the vertebrate neural retina. J. theor. Biol. 69 153 - 165
- MacDonald, N. (1978) Pattern formation in the amphibian retinotectal system. Ph.D. thesis, Edinburgh University
- Malmgren, L. and Olsson, Y. (1978) Sensitive method for histochemical demonstration of horseradish - peroxidase in neurons following retrograde axonal transport. Brain Res. 148 279 - 294
- Marchase, R.B., Barbera, A.J. and Roth, S. (1975) A molecular approach to retinotectal specificity. Ciba Foundation Symposium 29, pp 315 - 327
- Martin, K.A.C. (1978a) Combination of fibre-fibre competition and regional tectal differences accounting for the results of tectal graft experiments in goldfish. J. Physiol. 276 44P - 45P
- Martin, K.A.C. (1978b) D.Phil. thesis, University of Oxford
- Martin, K.A.C. and Perry, V. (1983) The role of fibre ordering and axon collateralization in the formation of topographic projections. Prog. Brain Res. 58 321 - 337
- Matthey, R. (1925) Recuperation de la vue apres resection des nerfs optiques chez le Triton. C. R. Seances Soc. Biol. Paris, 93, 904 - 906
- Maturana, H.R., Lettvin, J.Y., McCulloch, W.S. and Pitts, W.H. (1959) Evidence that cut optic nerve fibres in a frog regenerate to their proper places in the tectum. Science 130 1709 - 1710
- Maturana, H.R., Lettvin, J.Y., McCulloch, W.S. and Pitts, W.H. (1960) Anatomy and physiology of vision in the frog (*Rana pipiens*). J. gen. Physiol. 43 suppl. 2, pp 129 - 175

- McClay, D.R., Gooding, L.R. and Francon, M.E. (1977) A requirement for trypsin-sensitive cell-surface components for cell-cell interactions of embryonic neural retinal cells. J. Cell Biol. 75 56 - 66
- McLoon, S.C. (1982) Alterations in precision of the crossed retinotectal projection during chick development. Science 215 1418 - 1420
- McQuarrie, I.G. and Grafstein, B. (1981) Effect of a conditioning lesion on optic nerve regeneration in goldfish. Brain Res. 216 253 - 264
- Meek, J. (1981) The tectum mesencephali of the goldfish (Carassius auratus). A light and electron microscopic investigation. Nijmegen.
- Merrill, E.G. and Ainsworth, A. (1972) Glass-coated platinum-plated tungsten microelectrodes. Med. biol. Eng. 10 662 - 672
- Merzenich, M.M. and Kaas, J.H. (1980) Principles of organization of sensory perceptual systems in mammals. In: J.M. Sprague and A.N. Epstein (eds.) Progress in psychobiology and physiological psychology. Vol. 9 Academic press.N.Y.
- Mesulam, M.M. (1978) Tetramethyl benzidine for horseradish peroxidase histochemistry: a non-carcinogenic blue reaction product with superior sensitivity for visualising neural afferents and efferents. J. Histochem. Cytochem. 26 106 - 117
- Meyer, R.L. (1978) Evidence from thymidine labelling for continuing growth of retina and tectum in juvenile goldfish. Exp. Neurol. 59 99 - 111
- Meyer, R.L. (1979a) Retinotectal projection in goldfish to an inappropriate region with a reversal in polarity. Science 205 819 - 821
- Meyer, R.L. (1979b) "Extra" optic fibres exclude normal fibres from tectal regions in goldfish. J. comp. Neurol. 183 883 - 901

- Meyer, R.L. (1980) Mapping the normal and regenerating optic pathway of goldfish with autoradiographic methods. J.comp. Neurol. 189 273 - 289
- Meyer, R.L. (1982a) Tetrodotoxin blocks the formation of ocular dominance columns in goldfish. Science 218 589 - 591
- Meyer, R.L. (1982b) Ordering of retinotectal connections: a multivariate operational analysis. pp. 101 - 145 Current topics in developmental biology, vol. 17 ed. R.K. Hunt. Acad. Press.
- Meyer, R.L. (1983) Tetrodotoxin inhibits the formation of refined topography in goldfish. Dev. Brain Res. 6 293 - 298
- Meyer, R.L. (1984) Target selection by surgically misdirected optic fibres in the tectum of goldfish. J. Neurosci. 4 234 - 250
- Meyer, R.L. and Sperry, R.W. (1976) Retinotectal specificity: chemoaffinity theory. pp. 111 - 149 in: Neural and behavioural specificity: studies on the development of behaviour and the nervous system. Vol. 3. Ed. G. Gottlieb, Acad. Press, N.Y.
- Munro, N.S. and Beazley, L.D. (1982) Visuotectal projections following temporary transplantation of embryonic eyes to the body in Xenopus laevis. J. Embryol. exp. Morphol. 71 97 - 108
- Murphy, J.T., Kwan, H.C., MacKay, W.A. and Wong, Y.C. (1978) Spatial organization of pre-central cortex in awake primates. III. Input-output coupling. J. Neurophysiol. 41 1132 - 1140
- Murray, M. (1976) Regeneration of retinal fibres into the goldfish optic tectum. J. comp. Neurol. 168 175 - 193
- Murray, M. (1982) A quantitative study of regenerative sprouting by optic axons in goldfish. J. comp. Neurol. 209 352 - 362
- Murray, M. and Edwards, M.A. (1982) A quantitative study of the reinnervation of the goldfish optic tectum following optic nerve crush. J. comp. Neurol. 209 363 - 373

- Murray, M. and Forman, D.S. (1971) Fine structural changes in goldfish retinal ganglion cells during axonal regeneration. Brain Res. 32 287 - 298
- Murray, M. and Grafstein, B. (1969) Changes in the morphology and amino-acid incorporation of regenerating goldfish optic neurons. Exp. Neurol. 23 544 - 560
- Murray, M., Sharma, S.C. and Edwards, M.A. (1982) Target regulation of synaptic number in the compressed retinotectal projection of goldfish. J. comp. Neurol. 209 374 - 385
- Nieuwkoop, P.D. and Faber, J. A normal table of Xenopus laevis (Daudin). North Holland publ. co., Amsterdam
- Olsson, T.P., Forsberg, I. and Kristensson, K. (1978) Uptake and retrograde axonal transport of horseradish peroxidase in regenerating facial motor neurons of the mouse. J. Neurocytol. 7 323 - 336
- Pierce, M., Marchase, R.B. and Roth, S. (1978) The potential roles for ganglioside GM<sub>2</sub> and GM<sub>1</sub> synthetase in retinotectal specificity. pp. 261 - 274 in: S.E.B. Symposium XXXII Cell-cell recognition. Ed. A.S.G. Curtis CUP
- Prestige, M.C. and Willshaw, D.J. (1975) On a role for competition in the formation of patterned nerve connections. Proc. Roy. Soc. Lond. B 190 77 - 98
- Purves, D. and Lichtman, J.W. (1980) Elimination of synapses in the developing nervous system. Science 210 153 - 157
- Rager, G. (1980a) Development of topographic projections. Specificity of nerve connections by unspecific mechanisms? Trends in Neurosciences 3 43 - 45
- Rager, G. (1980b) Development of the retinotectal projection in the chicken. Adv. Anat. Embr. Cell Biol. 63

- Rager, G. and Rager, U. (1978) System - matching by degeneration. I. A quantitative electron microscopic study of the generation and degeneration of retinal ganglion cells in the chicken. Exp. Brain Res. 33 65 - 78
- Ramon y Cajal, S. (1893) The Teleost retina. pp. 787 - 805 in: "The vertebrate retina" Ed. Rodieck, R.W. W.H. Freeman and Co.
- Rankin, E.C.C. and Cook, J.E. (1984) Use of WGA-HRP to assess the progress of map refinement after regeneration of the goldfish optic nerve. J. Embryol. exp. Morph. 82 suppl. 233
- Rapaport, D.H. and Stone, J. (1982) The site of commencement of maturation in mammalian retina: observations in the cat. Dev. Brain Res. 5 273 - 279
- Reh, T.A. and Constantine-Paton, M. (1984) Retinal ganglion cell terminals change their projection sites during larval development of Rana pipiens. J. Neurosci. 4 442 - 457
- Reh, T.A., Pitts, E. and Constantine-Paton, M. (1983) The organization of the fibres in the optic nerve of normal and tectum-less Rana pipiens. J. comp. Neurol. 218 282 - 296
- Rho, J.H. and Hunt, R.K. (1980) Retinal projection to a surgically prepared "double lateral" tectum in Xenopus: electrophysiological studies. Dev. Biol. 80 436 - 453
- Rosene, D.L. and Mesulam, M.M. (1978) Fixation variables in horseradish peroxidase neuro-histochemistry: I. The effects of fixation time and perfusion procedures upon enzyme activity. J. Histochem. Cytochem. 26 28 - 39
- Scalia, F. and Arango, V. (1979) Topographic organization of the projection of the retina to the pretectal region in the rat. J. comp. Neurol. 186 271 - 292
- Scalia, F. and Arango, V. (1983) The anti-retinotopic organization of the frog's optic nerve. Brain Res. 266 121 - 126



- Scalia, F. and Fite, K. (1974) A retinotopic analysis of the central connections in the frog. J. comp. Neurol. 158 455 - 478
- Schmidt, J.T. (1978) Retinal fibres alter tectal position markers during the expansion of half retinal projections in goldfish. J. comp. Neurol. 177 279 - 299
- Schmidt, J.T. (1979) The laminar organization of optic nerve fibres in the tectum of goldfish. Proc. Roy. Soc. Lond. B 205 287 - 306
- Schmidt, J.T. (1982) The formation of retinotectal projections. Trends in Neurosciences 5 111 - 115
- Schmidt, J.T. (1983) Regeneration of the retinotectal following compression onto a half tectum in goldfish. J. Embryol. exp. Morphol. 77 39 - 51
- Schmidt, J.T., Cicerone, C.M. and Easter, S.S. (1978) Expansion of the half-retinal projection to the tectum in goldfish: an electrophysiological and anatomical study. J. comp. Neurol. 177 257 - 278
- Schmidt, J.T. and Edwards, D.L. (1983) Activity sharpens the map during the regeneration of the retinotectal projection in goldfish. Brain Res. 269 29 - 39
- Scholes, J.H. (1979) Nerve fibre topography in the retinal projection to the tectum. Nature 278 620 - 624
- Sharma, S.C. (1972a) Redistribution of visual projections in altered optic tecta of goldfish. Proc. natl. Acad. Sci. U.S.A. 69 2637 - 2639
- Sharma, S.C. (1972b) Reformation of retinotectal projections after various tectal ablations in adult goldfish. Exp. Neurol. 34 171 - 182
- Sharma, S.C. (1975) Visual projection in surgically created compound tectum in adult goldfish. Brain Res. 93 497 - 501

- Sharma, S.C. and Gaze, R.M. (1971) The retinotopic organisation of visual responses from tectal implants in adult goldfish. Arch. Ital. Biol. 109 357 - 366
- Sharma, S.C. and Hollyfield, J.G. (1978) Specification of central connections in Rana pipiens before appearance of post - mitotic ganglion cells. J. comp. Neurol. 155 395 - 408
- Sidman, A. (1960) Histiogenesis of the mouse retina studied with thymidine-<sup>3</sup>H. pp.487 - 506 In: The structure of the eye. G.K. Smelser, ed. Academic Press
- Siegel, A., Edinger, H. and Ohgami, S. (1974) The topographical organization of the hippocampal projection to the septal area: a comparative neuroanatomical analysis in the gerbil, rat, rabbit and cat. J. comp. Neurol. 157 359 - 378
- Sperry, R.W. (1943a) Visuomotor coordination in the newt (Triturus viridescens) after regeneration of the optic nerve. J. comp. Neurol. 79 33 - 35
- Sperry, R.W. (1943b) Effect of 180 degree rotation of the retinal field on visuomotor coordination. J. exp. Zool. 92 263 - 279
- Sperry, R.W. (1944) Optic nerve regeneration with return of vision in anurans. J. Neurophysiol. 7 57 - 69
- Sperry, R.W. (1945) Restoration of vision after crossing of optic nerves and after contralateral transplantation of eye. J. Neurophysiol. 8 15 - 28
- Sperry, R.W. (1948) Patterning of central synapses in regeneration of the optic nerve in Teleosts. Physiol. Zool. 21 351 - 361
- Sperry, R.W. (1951) Mechanisms of neural maturation. pp. 236 - 280 in: Handbook of experimental psychology. Ed. S.S. Stevens. Wiley and Sons, N.Y.

- Sperry, R.W. (1963) Chemoaffinity in the orderly growth of nerve fibre patterns and connections. Proc. natl. Acad. Sci. U.S.A. 50 703 - 710
- Springer, A.D. (1981) Normal and abnormal retinal projections following the crush of one optic nerve in goldfish (Carassius auratus). J. comp. Neurol. 199 87 - 95
- Springer, A.D. and Agranoff, B.W. (1977) Effect of temperature on goldfish optic nerve regeneration: a radioautographic and behavioural study. Brain Res. 128 405 - 415
- Springer, A.D., Heacock, A.M., Schmidt, J.T. and Agranoff, B.W. (1977) Bilateral tectal innervation by regenerating optic nerve fibres in goldfish: a radioautographic, electrophysiological and behavioural study. Brain Res. 128 417 - 427
- Steedman, J.G., Stirling, R.V. and Gaze, R.M. (1979) The central pathways of optic fibres in Xenopus tadpoles. J. Embryol. exp. Morphol. 50 199 - 215
- Stirling, R.V. and Merrill, E.G. (1984) How retinal axons grow in the tectum: branching analysis of axonal arbors of single, physiologically identified frog retinal axons. J. Embryol. exp. Morph. 82 suppl. 236
- Stone, L.S. (1949) Regeneration of the neural portion of the retina from pigment cells in adult Urodele eyes. Proc. Soc. exp. Biol. Med. 72 382 - 384
- Straus, W. (1982) Imidazole increases the sensitivity of the cytochemical reaction for peroxidase with diaminobenzidine at neutral pH. J. Histochem. Cytochem. 30 491 - 493
- Straznicky, C. and Gaze, R.M. (1971) The growth of the retina in Xenopus laevis: an autoradiographic study. J. Embr. exp. Morphol. 26 57 - 69

- Straznicky, C. and Gaze, R.M. (1972) The development of the tectum in Xenopus laevis: an autoradiographic study. J. Embryol. exp. Morphol. 28 87 - 115
- Straznicky, C. and Gaze, R.M. (1980) Stable programming for map orientation in fused eye fragments in Xenopus. J. Embryol. exp. Morphol. 55 123 - 142
- Straznicky, C., Gaze, R.M. and Horder, T.J. (1979) Selection of appropriate medial branch of the optic tract by fibres of ventral retinal origin during development and in regeneration: an autoradiographic study in Xenopus. J. Embryol. exp. Morph. 50 253 - 267
- Straznicky, K., Gaze, R.M. and Keating, M.J. (1971) The retinotectal projection after uncrossing the optic chiasma in Xenopus with one compound eye. J. Embryol. exp. Morph. 26 523 - 542
- Straznicky, K., Gaze, R.M. and Keating, M.J. (1974) The retinotectal projection from a double - ventral compound eye in Xenopus laevis. J. Embryol. exp. Morph. 31 123 - 137
- Stuermer, C.A.O. (1984) Rules for retinotectal terminal arborizations in the goldfish optic tectum: a whole-mount study. J. comp. Neurol. 229 214 - 232
- Stuermer, C.A.O. and Easter, S.S. (1984) A comparison of the normal and regenerated retinotectal pathways of the goldfish. J. comp. Neurol. 223 57 - 76
- Swinscow, T.D.V. (1980) Statistics at square one. 7th ed. British Medical Association.
- Talbot, S.A. and Marshall, W.H. (1941) Physiological studies on neural mechanisms of visual location and discrimination. Am. J. Ophthalm. 24 1255 - 1264

- Taylor, J., Willshaw, D.J. and Gaze, R.M. (1984) The pathways of fibres from translocated eyes in *Xenopus*. J. Embryol. exp. Morph. 82 suppl. 237
- Thanos, S. and Bonhoeffer, F. (1983) Investigations on the development and topographic order of retinotectal axons: anterograde and retrograde staining of axons and perikarya with rhodamine in vivo. J. comp. Neurol. 219 420 - 430
- Tosney, K., Hoskins, S. and Hunt, R.K. (1978) Testing a radial model for positional information in the development of frog retinotectal specificity. Biophys. J. 21 110a
- Tyrer, N.M. and Altman, J.S. (1974) Motor and sensory flight neurones in a locust demonstrated using cobalt chloride. J. comp. Neurol. 157 117 - 138
- Udin, S.B. (1978) Permanent disorganization of the regenerating optic tract in the frog. Exp. Neurol. 58 455 - 470
- Udin, S.B. and Fisher, M.D. (1983) Visualization of HRP-filled axons in unsectioned, flattened optic tecta in goldfish. J. Neurosci. Methods 9 283 - 285
- Watson, W.E. (1968) Observations on the nucleolar and total cell body nucleic acid of injured nerve cells. J. Physiol. 196 655 - 676
- Weiss, P.A. (1941) Nerve patterns: the mechanisms of nerve growth. Growth, third symposium, 5 163 - 208
- Westerman, R.A. (1965) Specificity in regeneration of optic and olfactory pathways in teleost fish. In: Studies in physiology. Eds. D.R. Curtis and A.K. McIntyre. Pub.: Springer.
- Wigglesworth, V.B. (1959) "The control of growth and form: a study of the epidermal cell of an insect." Oxford.
- Willshaw, D.J., Fawcett, J.W. and Gaze, R.M. (1983) The visuotectal projections made by *Xenopus* "pie - slice" compound eyes. J. Embryol. exp. Morph. 74 29 - 45

- Willshaw, D.J. and von der Malsburg, C. (1976) How patterned nerve connections can be set up by self - organization. Proc. roy. Soc. Lond. B 194 431 - 445
- Willshaw, D.J. and von der Malsburg, C. (1979) A marker induction mechanism for the establishment of ordered neural mappings: its application to the retinotectal problem. Phil. Trans. roy. Soc. B 287 203 - 243
- Yeziarski, R.P. and Bowker, R.M. (1981) A retrograde double-label tracing technique using horseradish peroxidase and the fluorescent dye 4', 6'-diaminido-2-phenylindole dihydrochloride (D.A.P.I.) J. Neurosci. Methods 4 53 - 62
- Yoon, M.G. (1971) Reorganization of the retinotectal projection following surgical operation on the optic tectum in adult goldfish. Exp. Neurol. 33 395 - 411
- Yoon, M.G. (1972a) Reversibility of the reorganisation of the retinotectal projection in goldfish. Exp. Neurol. 35 565 - 577
- Yoon, M.G. (1972b) Transposition of the visual projection from the nasal hemiretina on to the foreign rostral zone of the tectum in goldfish. Exp. Neurol. 37 451 - 462
- Yoon, M.G. (1973) Retention of original topographic polarity by the 180 degree rotated tectal reimplant in young adult goldfish. J. Physiol. 233 575 - 588
- Yoon, M.G. (1975) Topographic polarity of the optic tectum studied by reimplantation of the tectal tissue in adult goldfish. In: Cold Spring Harbour Symposia on Quantitative Biology. 40 The synapse.
- Yoon, M.G. (1976) Progress of topographic regulation of the visual projection in the halved optic tectum in adult goldfish. J. Physiol. 257 621 - 643
- Yoon, M.G. (1977) Retinotectal projection after translocation of two tectal grafts within the same optic tectum in goldfish. Soc. for Neurosci., Abstracts 3 1233

ADDENDA TO BIBLIOGRAPHY:

- Cook, J. E. and Rankin, E. C. C. (1984a) An effect of stroboscopic light on the retinotopography of regenerated optic terminals in goldfish. J. Physiol. (in press) 28P
- Gaze, R.M. and Keating, M. J. (1972) The visual system and "neuronal specificity". Nature, 237 375 - 8
- Horder, T.J. and Martin, K.A.C. (1982) Some determinants of optic terminal localization and retinotopic polarity within fibre populations in the tectum of goldfish. J. Physiol. 333 481 - 509
- Marchase, R.B. (1977) Biochemical investigations of retinotectal adhesive specificity. J. Cell Biol. 75 237 - 257
- Straznicky, C. and Gaze, R.M. (1982) The innervation of a virgin tectum by a double temporal or double nasal eye in Xenopus J. Embryol. exp. Morph. 68 9 - 21
- Straznicky, K., Gaze, R.M. and Keating, M.J. (1981) The development of the retinotectal projections from compound eyes in Xenopus. J. Embryol. exp. Morph. 62 13 - 35
- Weiler, I.J. (1966) Restoration of visual acuity after optic nerve section and regeneration, in Astronotus ocellatus. Expl. Neurol. 15 377 - 386

## APPENDIX

Proof that axons in the dorsal part of the optic nerve, adjacent to the sclera, belong to central ganglion cells has been published in Dawney (1979b, 1981b) and Easter, Rusoff and Kish (1981).

I reproduce here a specimen fully described in the first of these papers, with the generous permission of its author.

A cut was made through the juxta-scleral part of the left optic nerve of a normal goldfish, severing only dorsal fibres. Into this lesion a crystal of Horseradish Peroxidase (HRP) was inserted. Visuotectal mapping immediately after this lesion revealed a central absolute scotoma (figure 92).

Subsequent histochemical processing showed that HRP had labelled central ganglion cells (plate 10).

Lesions produced by myself using a similar technique produced a similar pattern of labelling (plate 11).



D66725/86

UNRESTRICTED

ANALYSIS OF RETINOTECTAL  
REGENERATION IN GOLDFISH  
USING POLAR DIMENSIONS:  
TEMPORAL SEQUENCE AND SPATIAL ORDER

DAVID COLIN MANSFIELD B.A.

SUBMITTED FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY  
OF THE OPEN UNIVERSITY

DEPARTMENT OF BIOLOGY

Date of Submission: March 1985  
Date of Award: 15.10.85

MARCH 1985

VOLUME TWO

FIGURES AND PLATES

LEGENDS PRECEDE THE FOLLOWING GROUPS OF FIGURES:

1, 2 - 17, 18 - 27, 28- 29, 30, 31, 32 - 37, 38 - 49

as a group and individually, 50 - 78, 79 - 91, 92.

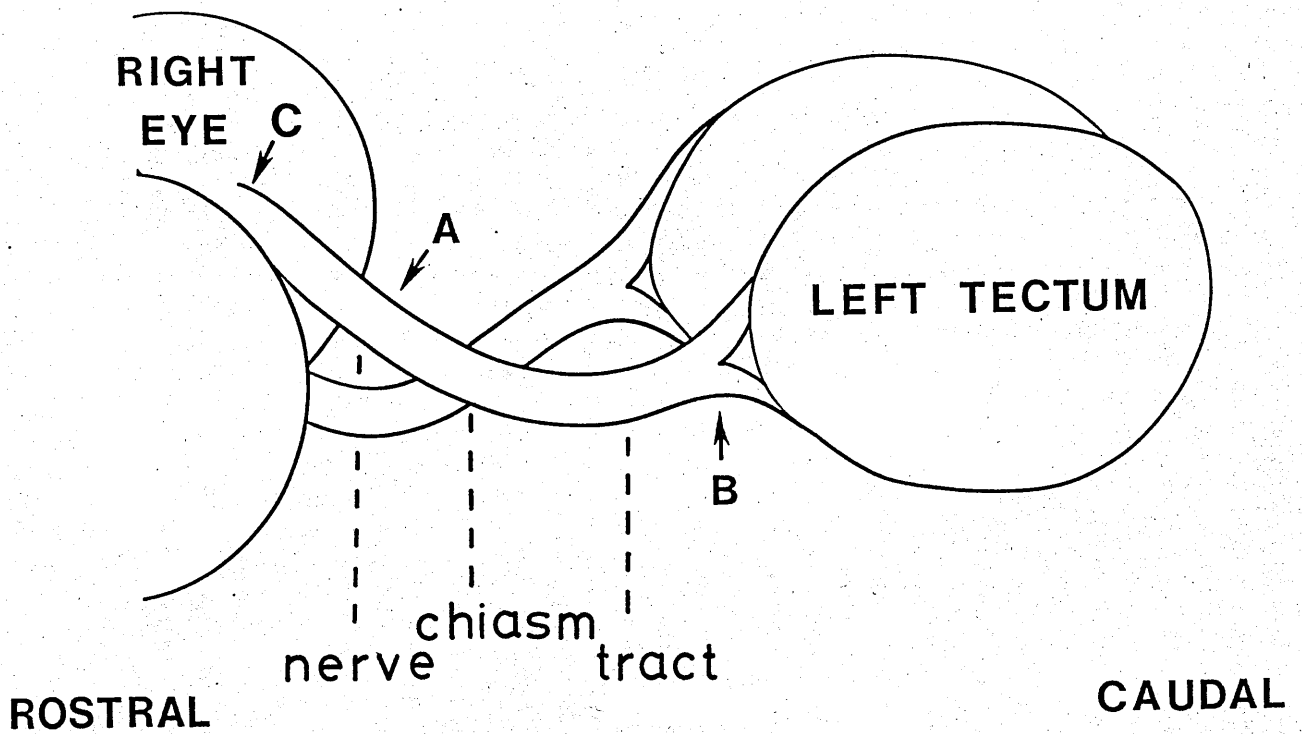
Relevant pages of text are cited.

FIGURE 1

Schematized view of the gross anatomy of both retino-tectal  
projections of the goldfish.

Text page 6.

## GOLDFISH OPTIC PATHWAY



A,B,C = LESION SITES (SEE TEXT)

FIGURE 1

## FIGURES 2 - 17

Drawings of flat-mounted goldfish neural retinae, each from the right eye. Dorsal uppermost, nasal to the right.

The boundary shows the edge of the retina at the ora serrata, with radial incisions into the retina at ventral, dorsal, nasal and temporal poles.

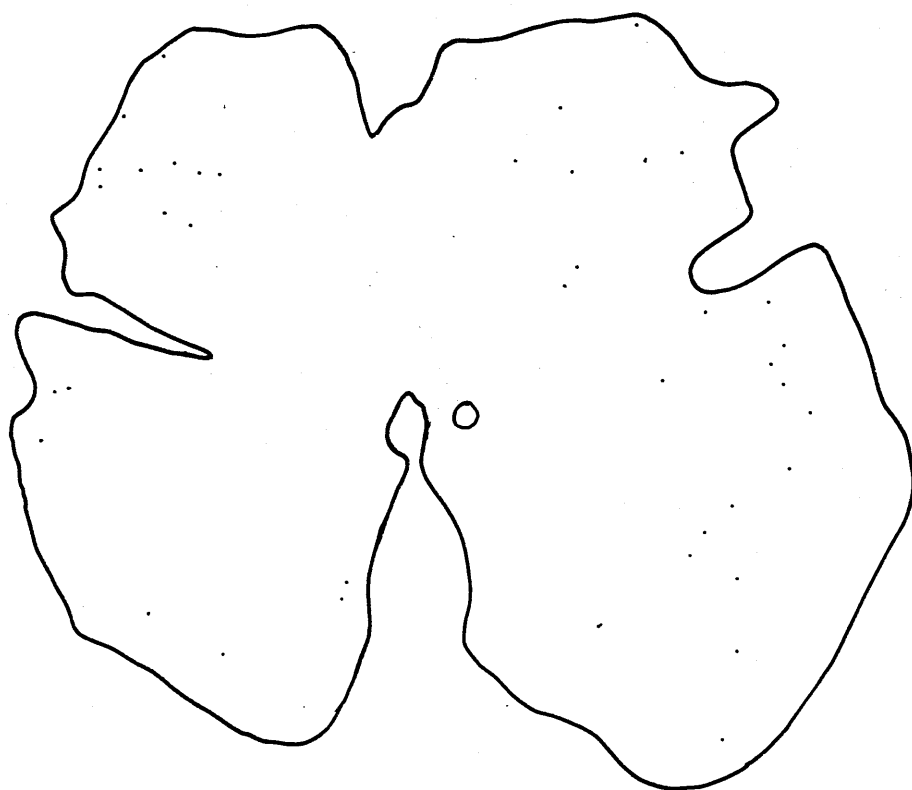
The central ring indicates the position of the optic nerve head.

Dots show the positions of microscopically identified ganglion cells stained with Horseradish Peroxidase. Text page 45.

The duration of regeneration shown under each of figures 2 - 16 shows the number of days between mid-orbital nerve transection (page 38) and application of HRP to the optic tract (page 39).

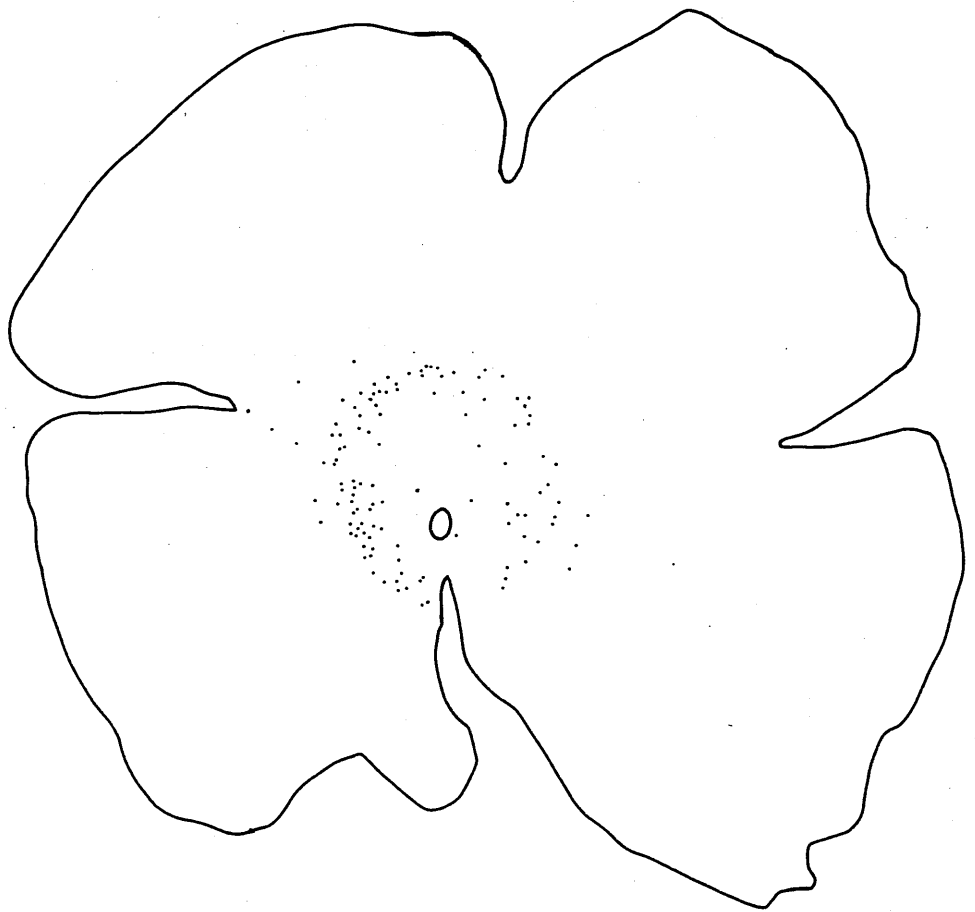
Figure 17 shows a specimen labelled without prior transection of the optic nerve.

These figures are described and discussed on text pages 50 - 63.



22d Regeneration

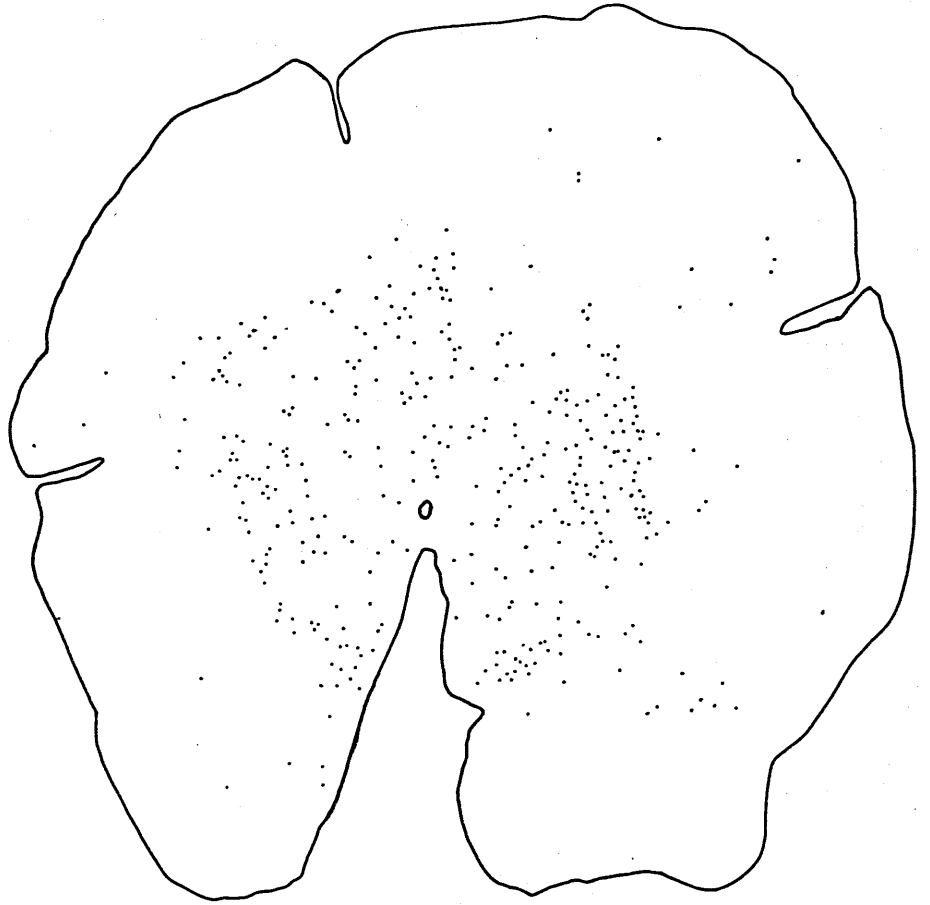
FIGURE 2



22d Regeneration

FIGURE 3





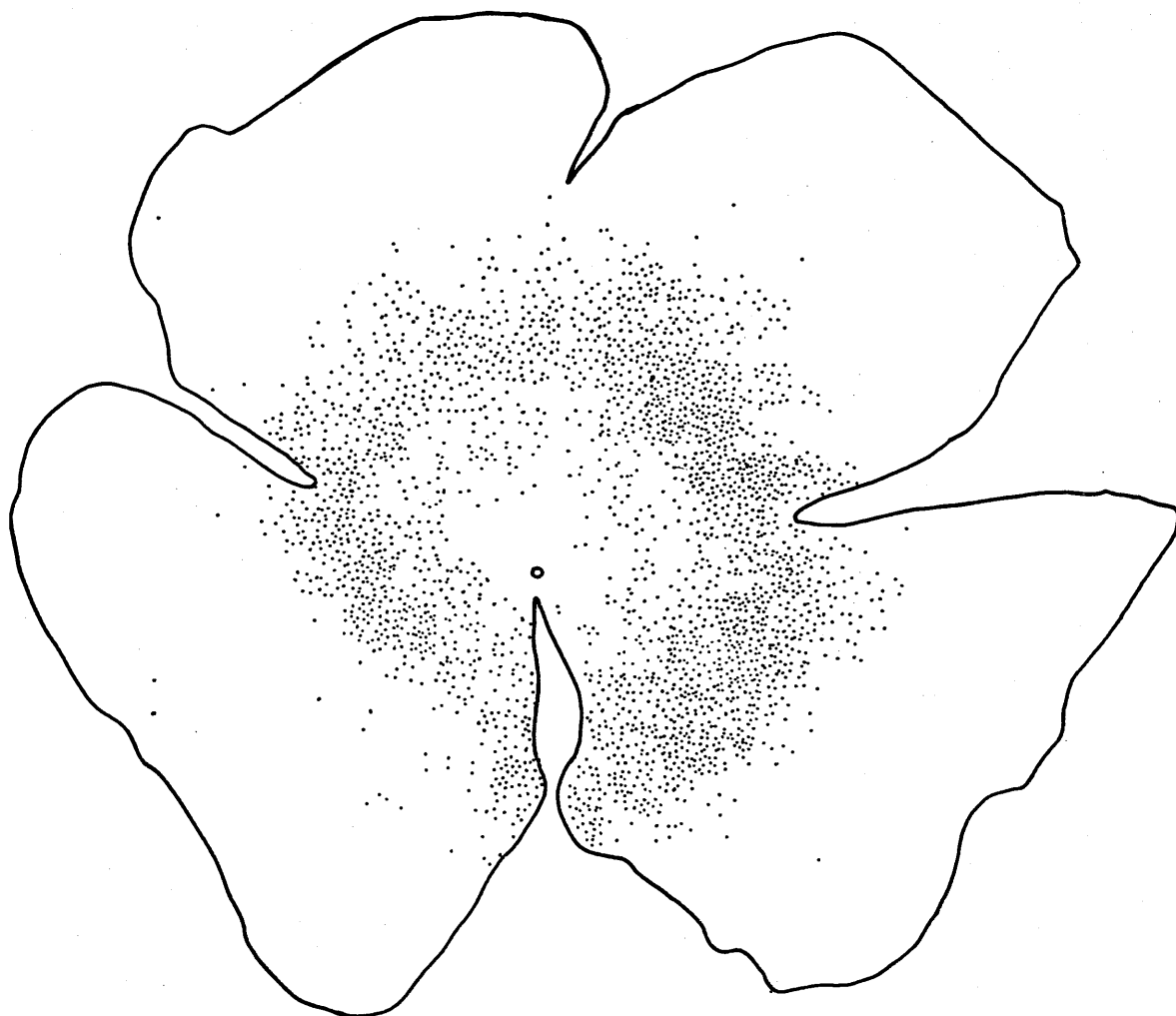
26d Regeneration

FIGURE 4



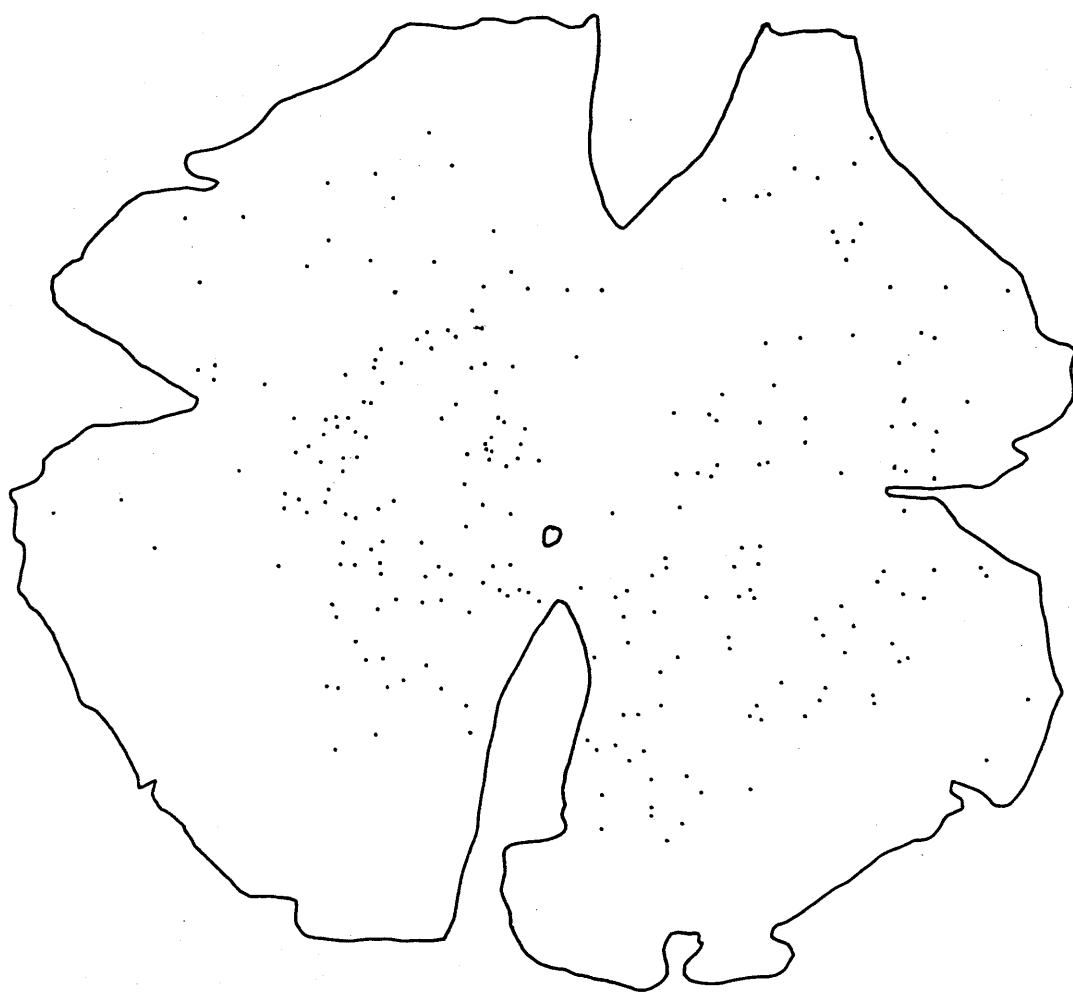
26 d    Regeneration

FIGURE 5



26d Regeneration

FIGURE 6



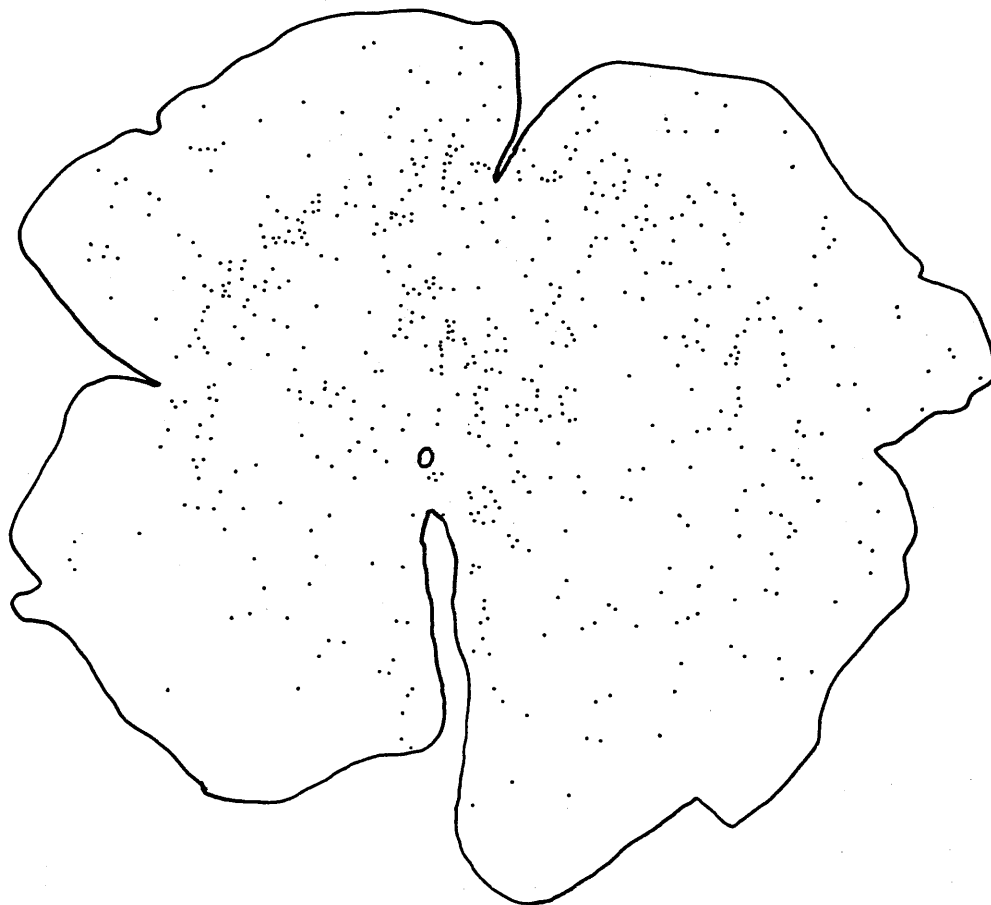
26d Regeneration

FIGURE 7



36d Regeneration

FIGURE 8



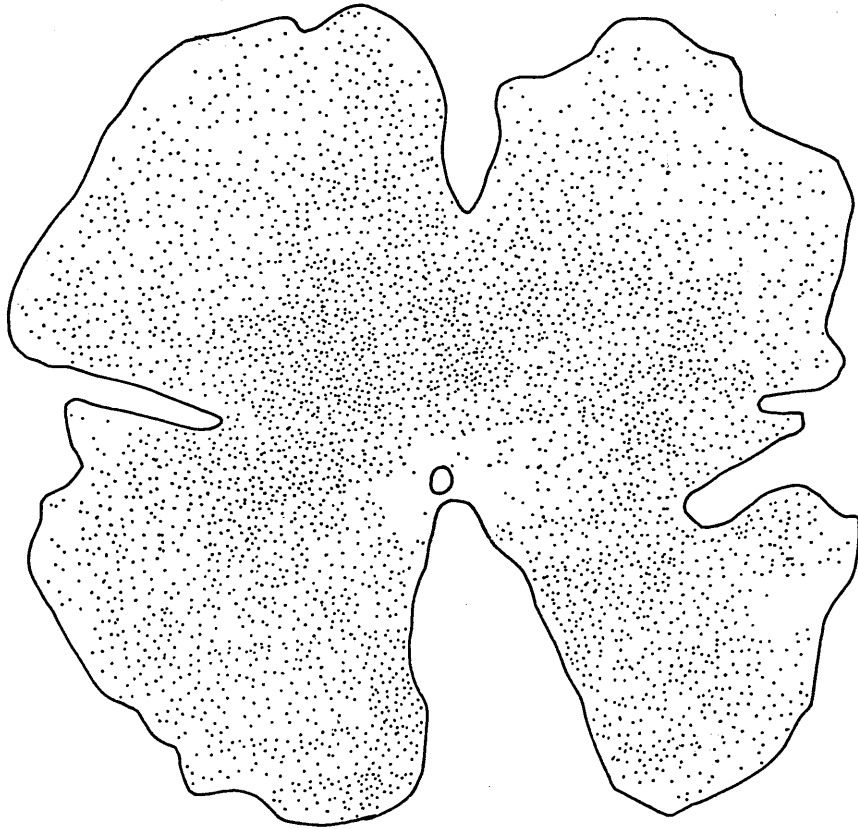
36d Regeneration

FIGURE 9



36d Regeneration

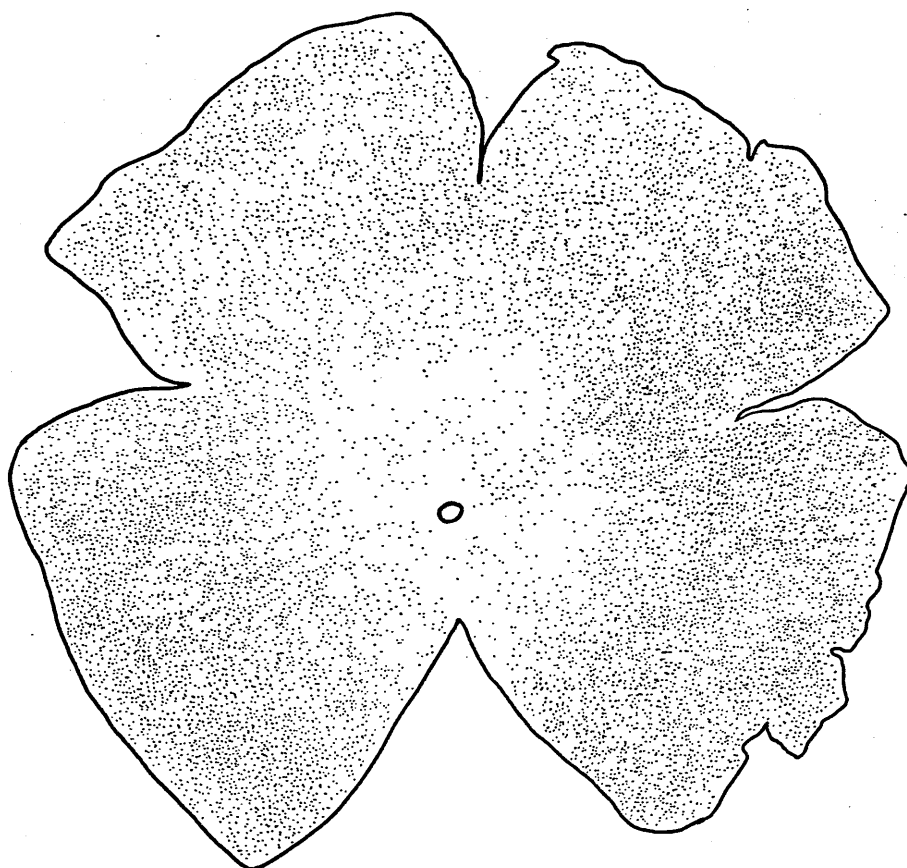
FIGURE 10



48 d    Regeneration

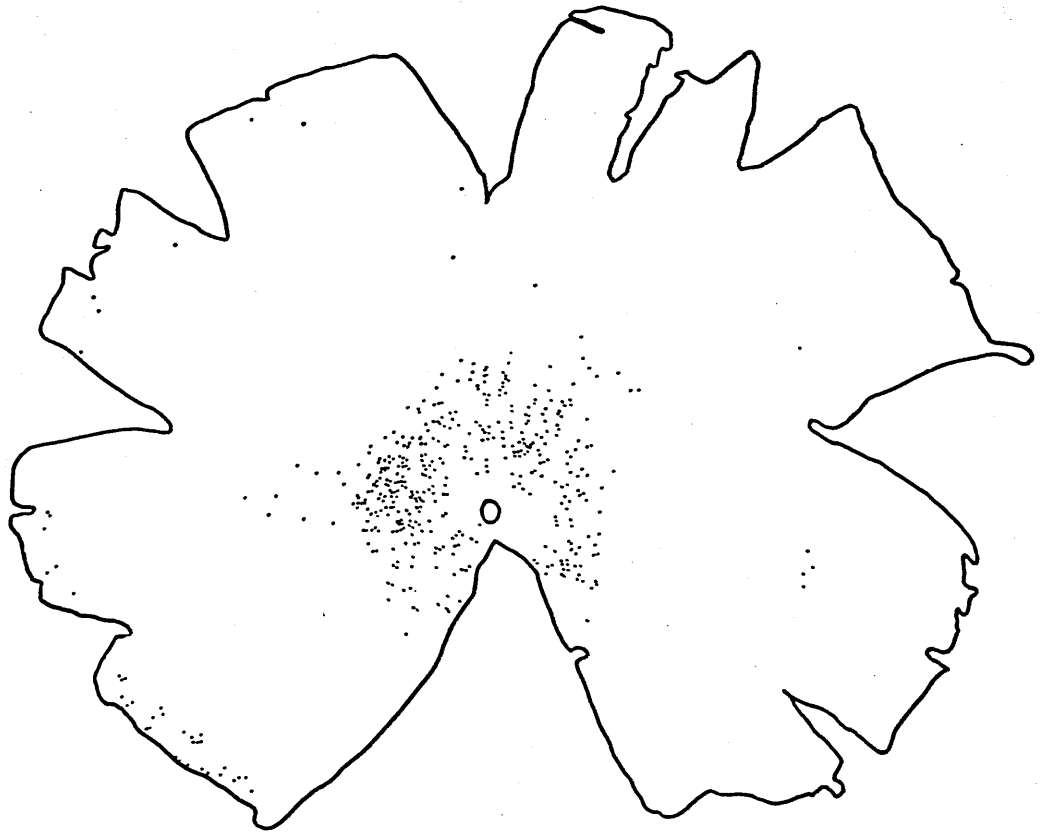
FIGURE 11





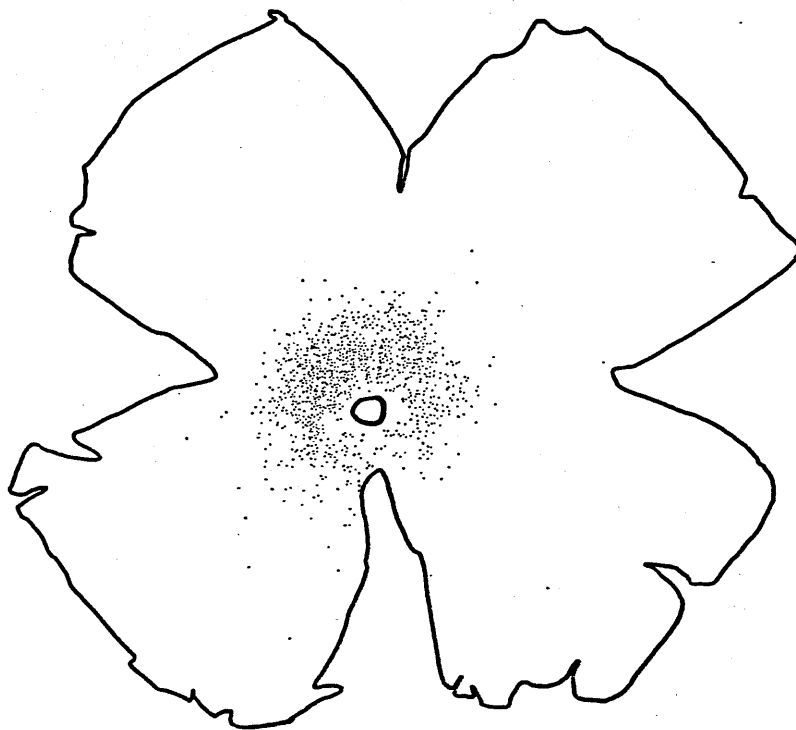
84d Regeneration

FIGURE 12



14d Regeneration

FIGURE 13



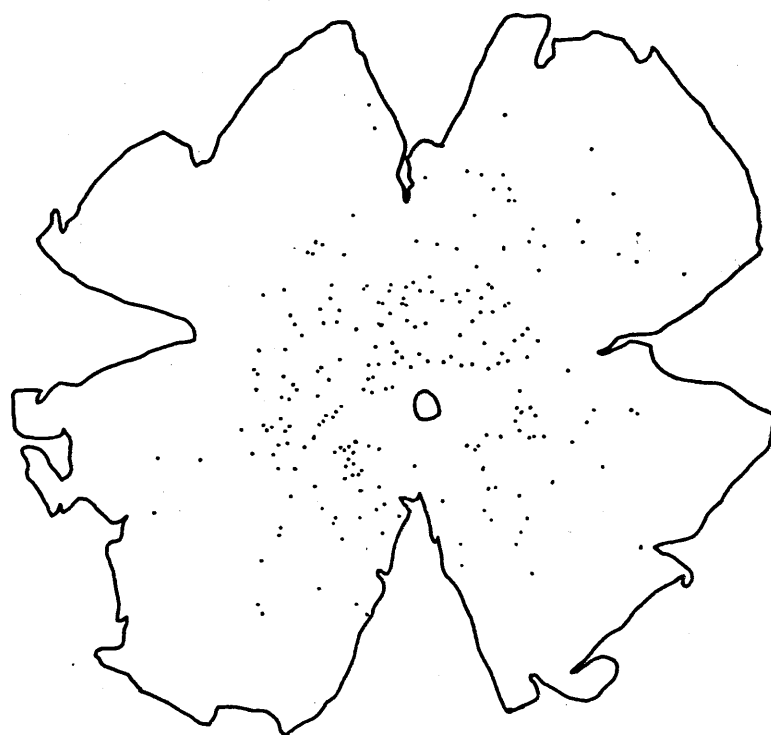
16d Regeneration

FIGURE 14



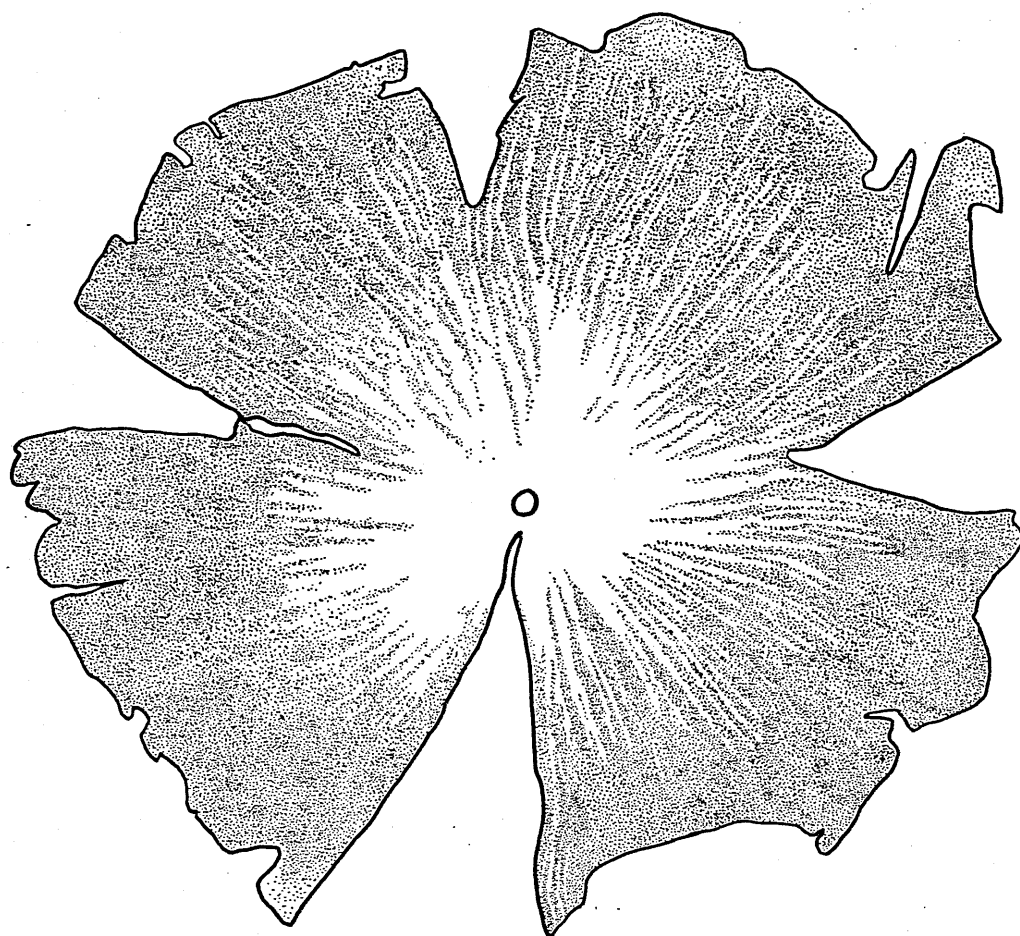
16d Regeneration

FIGURE 15



18d Regeneration

FIGURE 16



NORMAL

FIGURE 17

# FIGURES 18 - 27

**N** = Number of cresyl violet-stained ganglion cells within one rectangular field of view of the microscope using a x40 objective.

**D** = Distance from the optic nerve head to the lateral extremity of the field of view expressed as a multiple of one field of view using a x40 objective,

Methods: page 46. Discussion: page 62

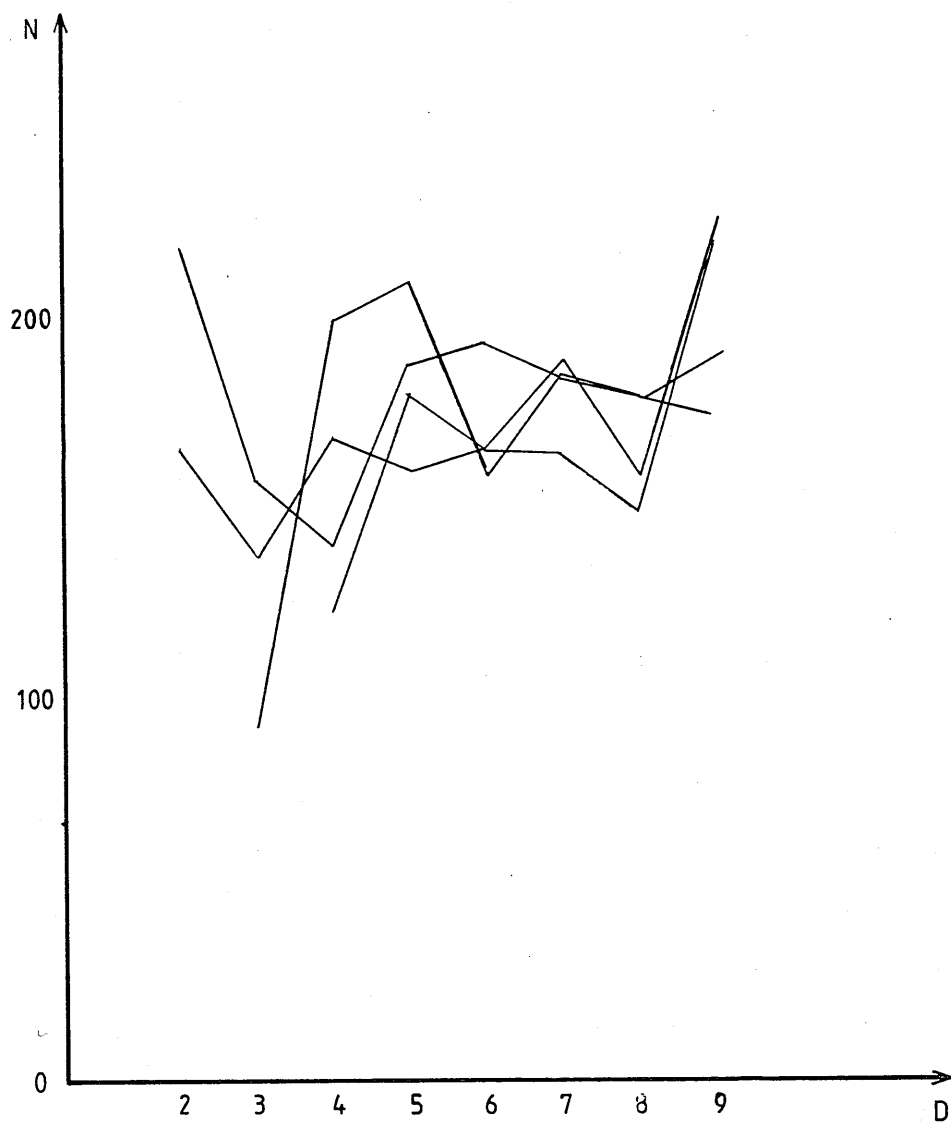
Each angulated line links observations along one radius of the retina. Four radii were selected at intervals of about 90°.

Figure ... refers to retina in figure ..

18	-
19	3
20	4
21	6
22	8
23	10
24	12

The duration of regeneration of regeneration indicated below each figure is the sum of regeneration prior to, and after, application of HRP to the optic tract.

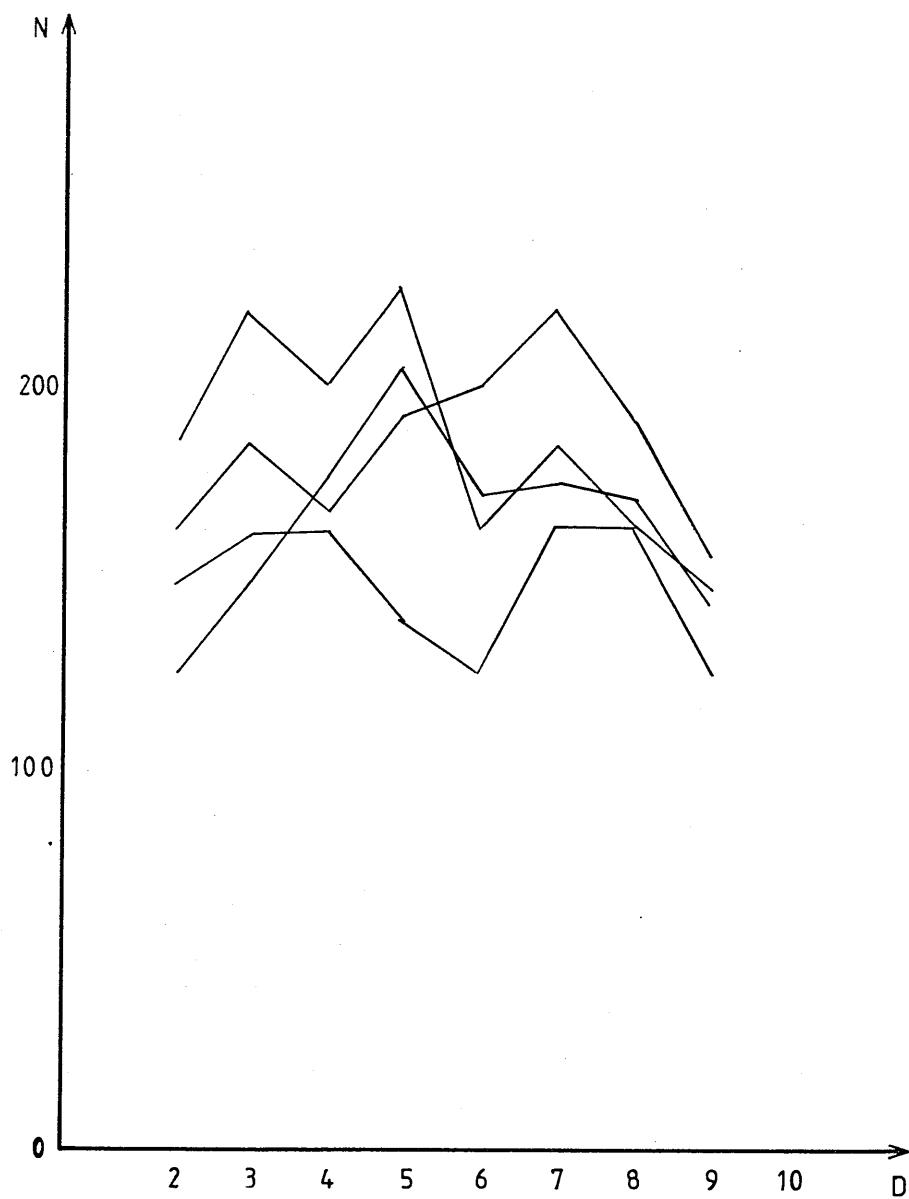
Figures 25 - 27 show counts made on three normal retinae.



22days Regeneration

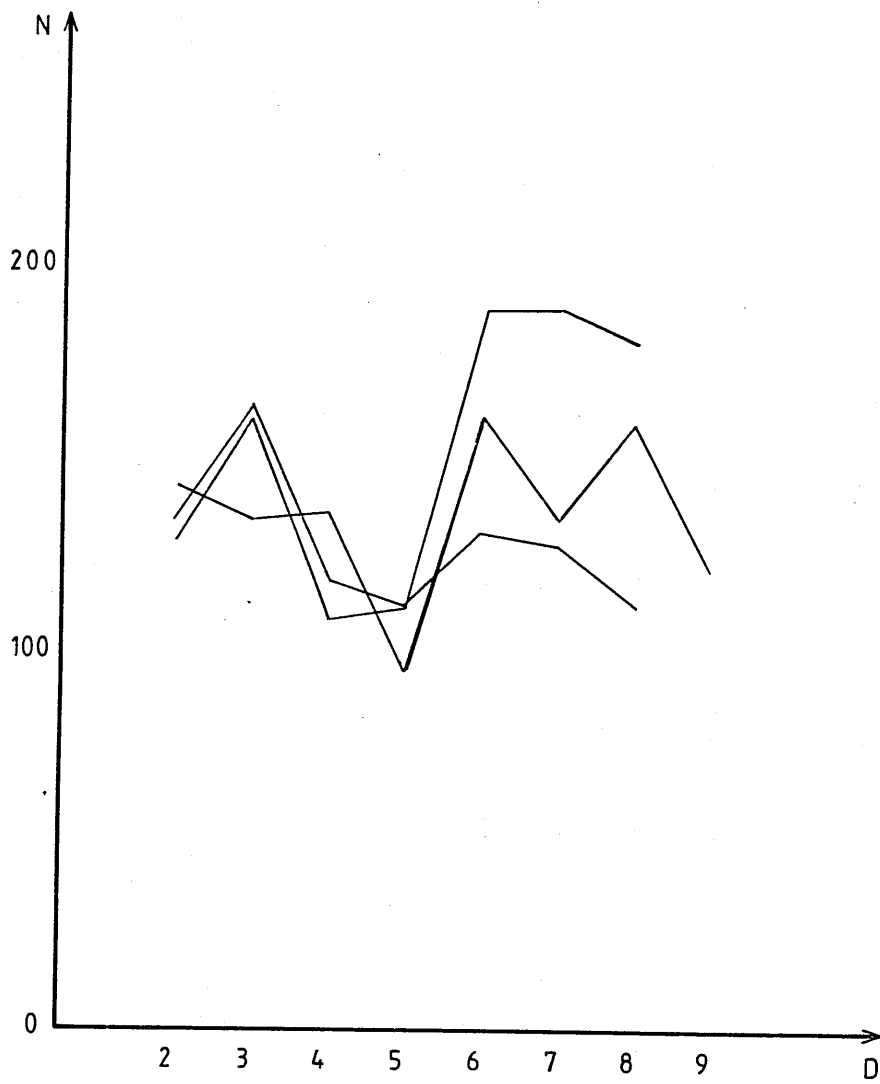
FIGURE 18





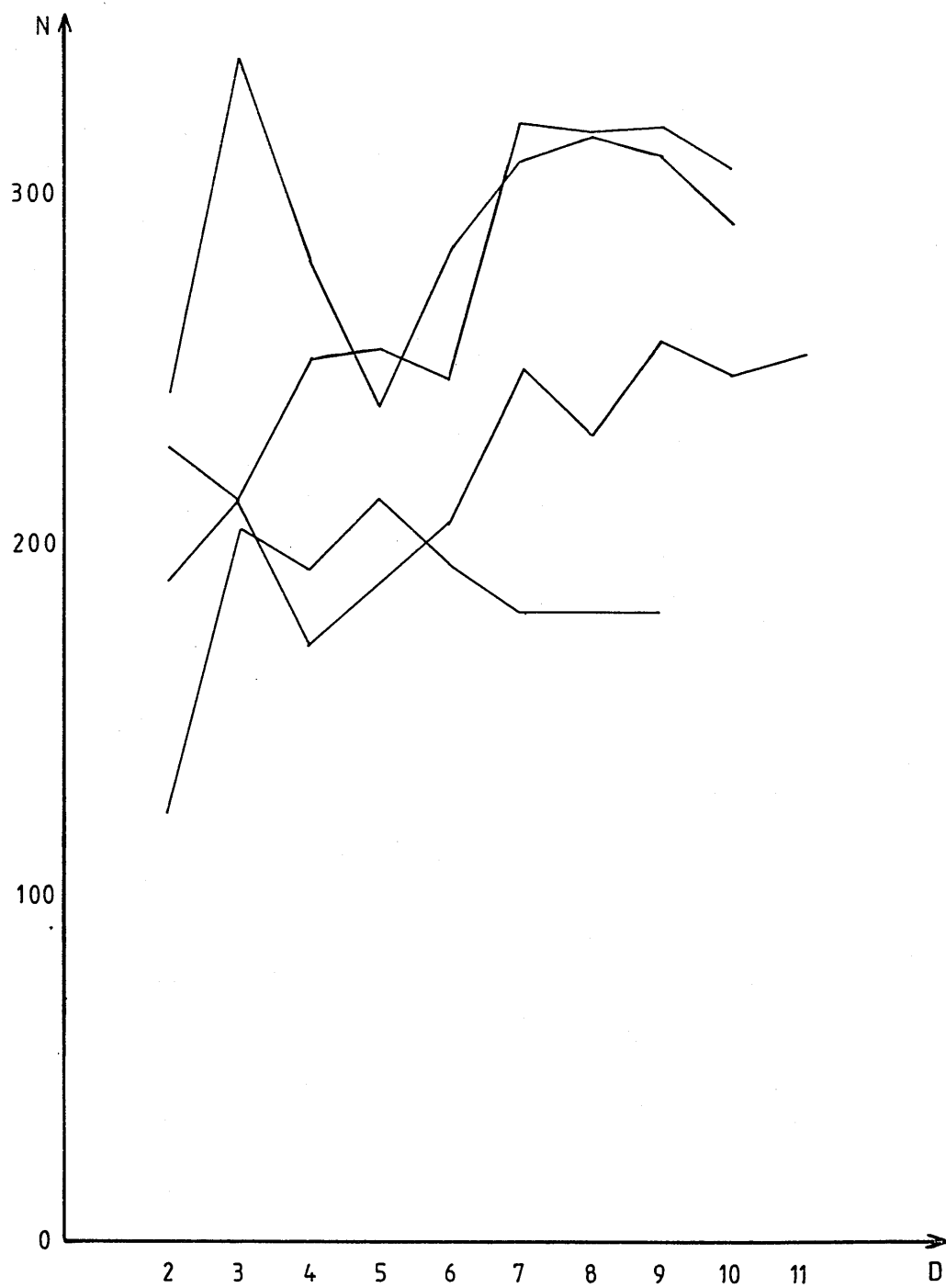
27 days

FIGURE 19



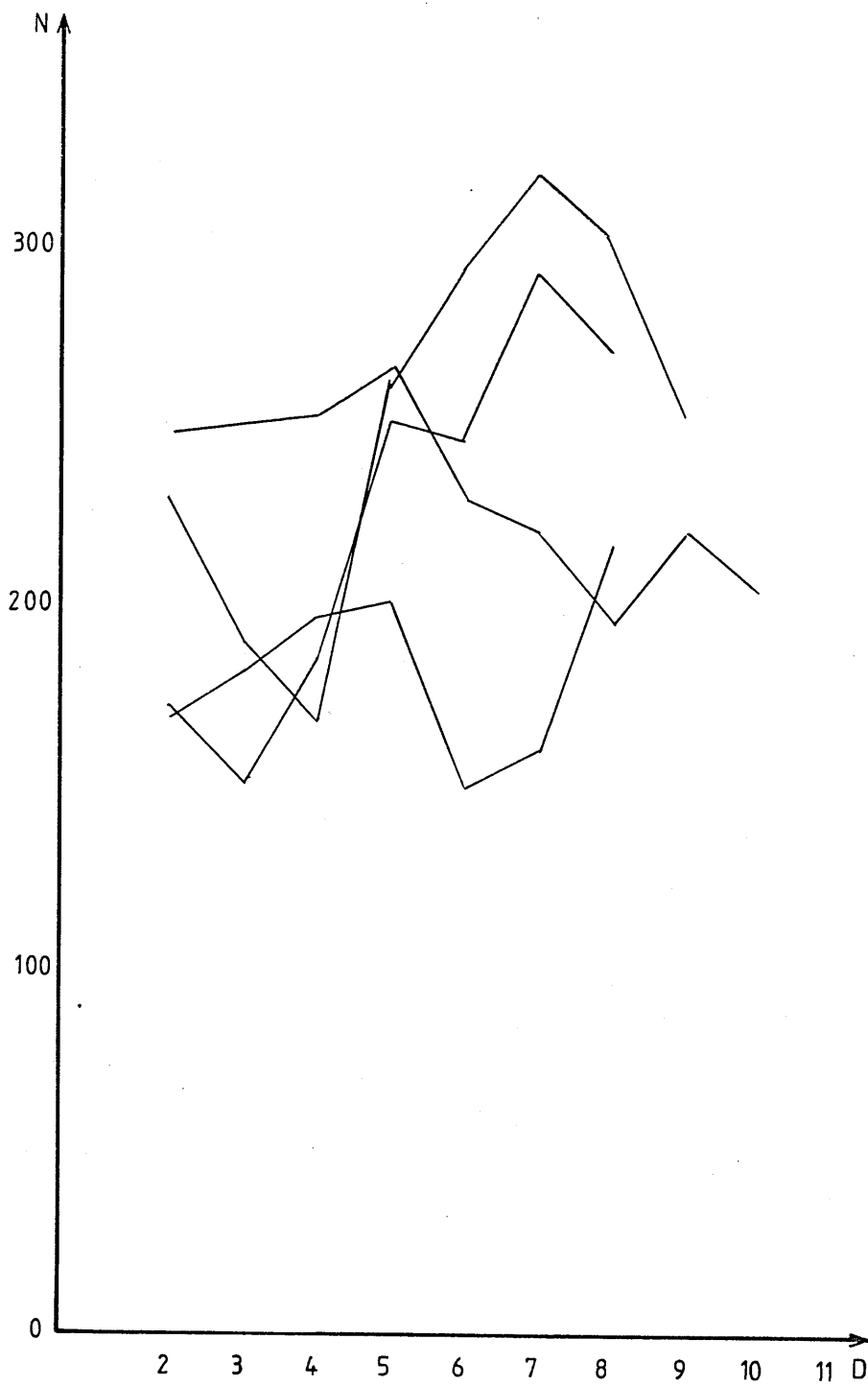
31 days

FIGURE 20



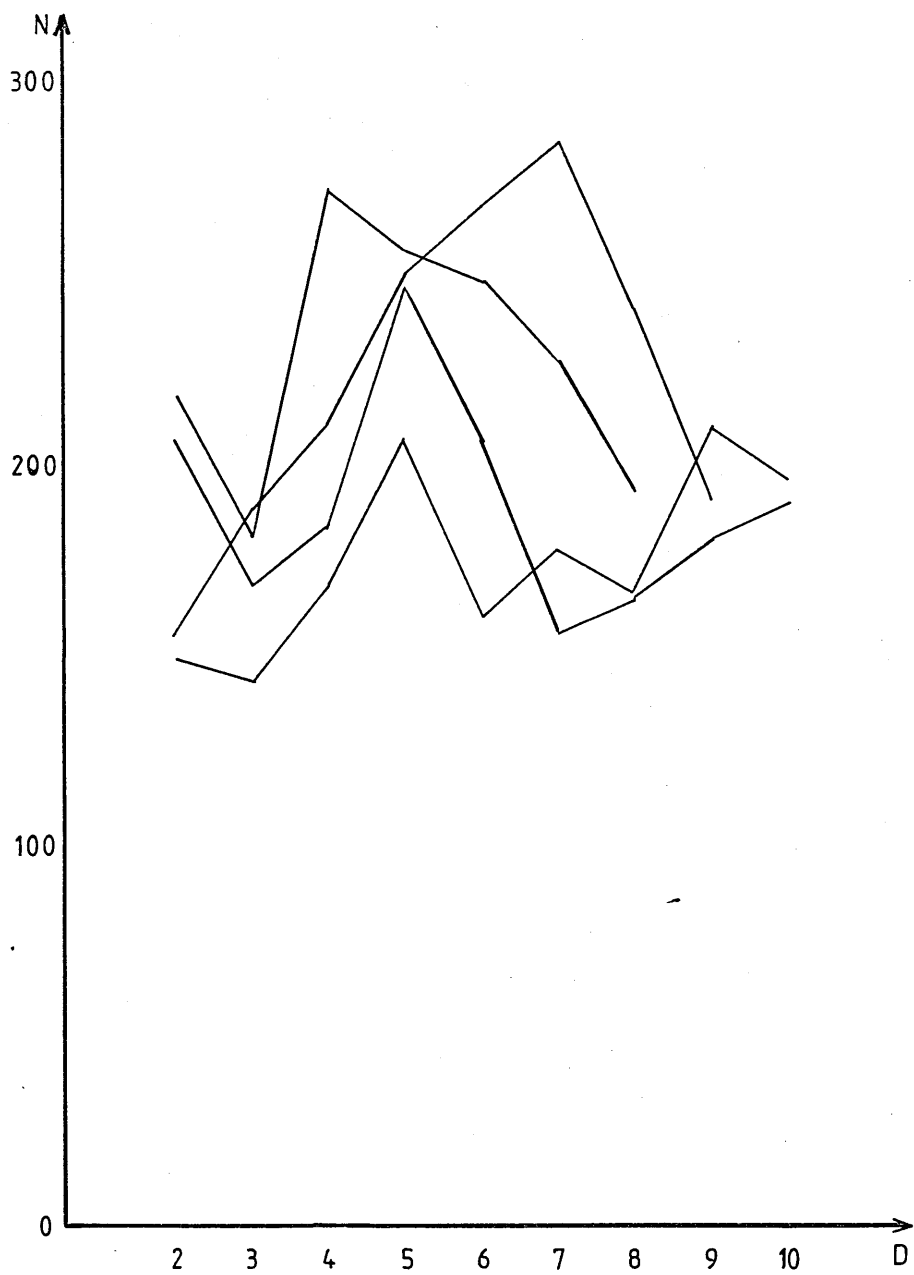
31 days

FIGURE 21



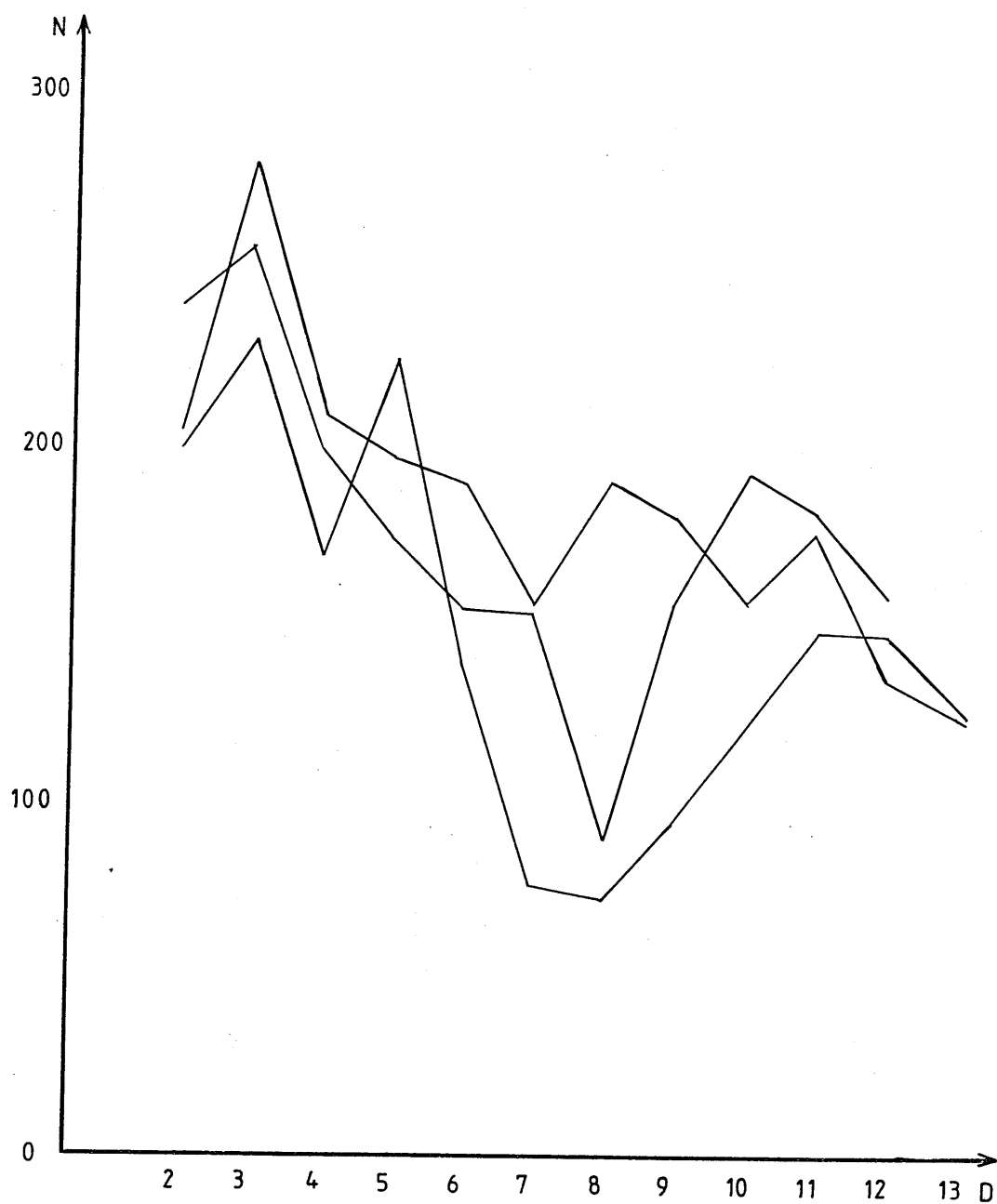
38 days

FIGURE 22



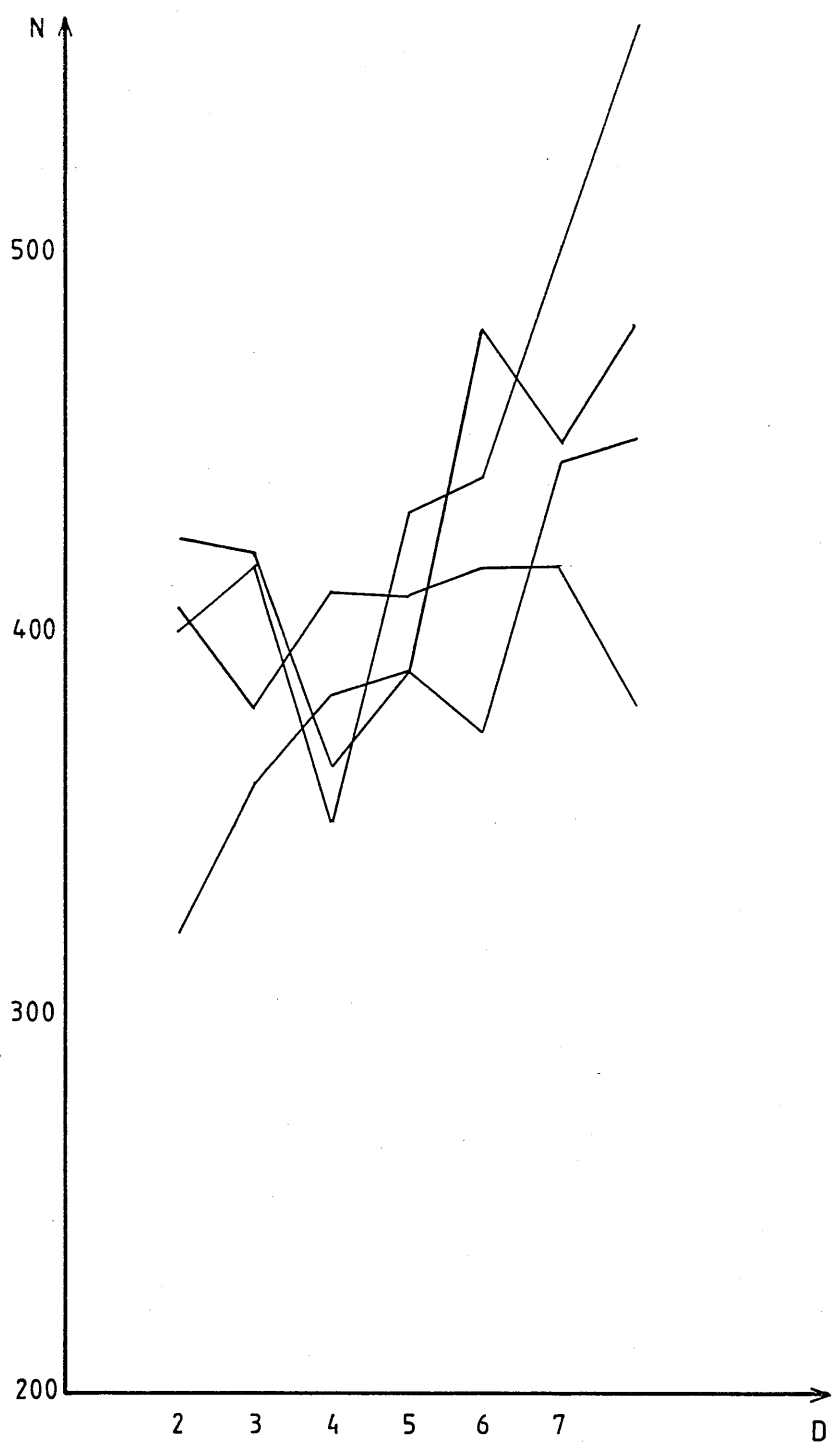
44 days

FIGURE 23



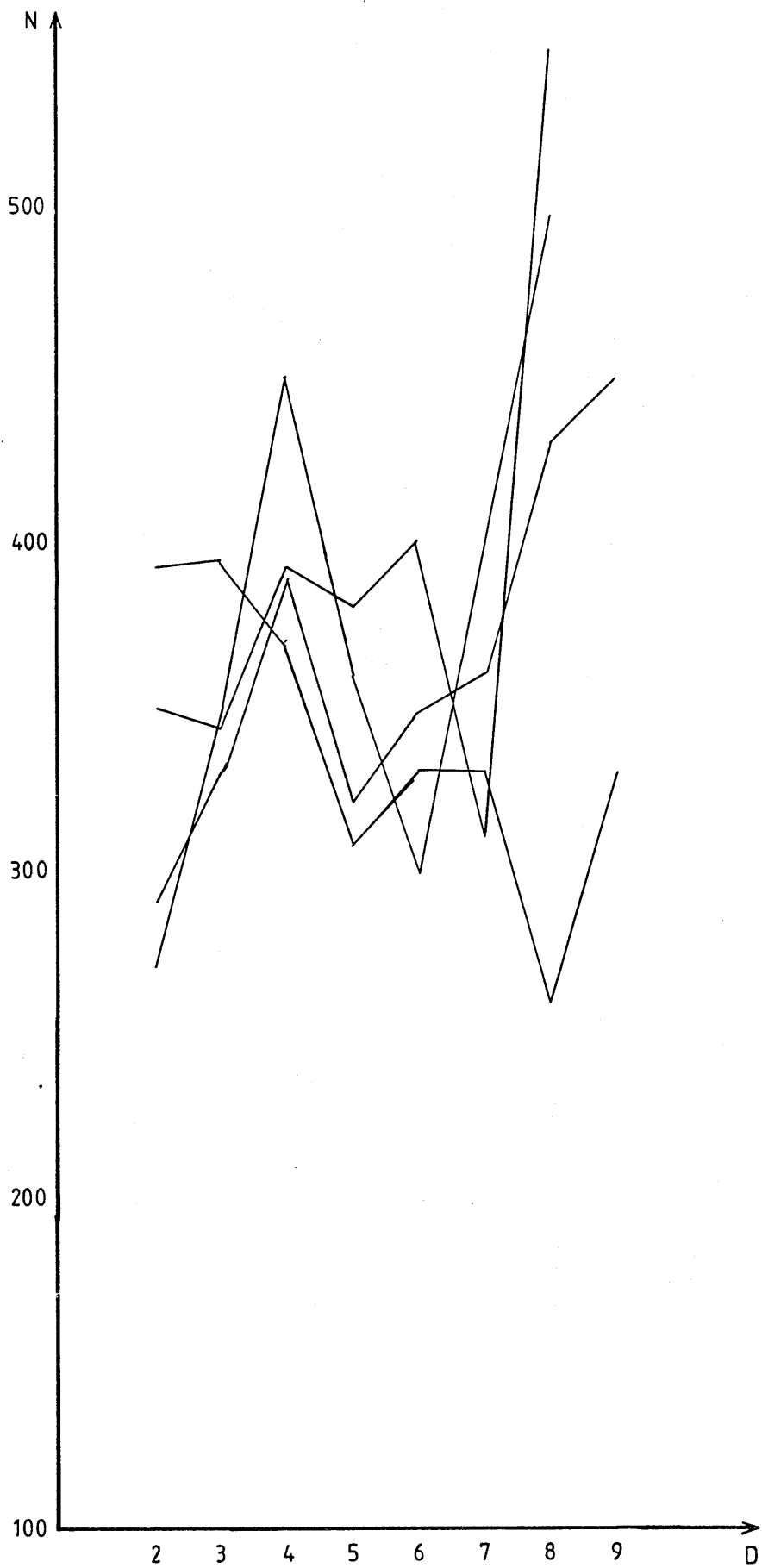
89 days

FIGURE 24



NORMAL

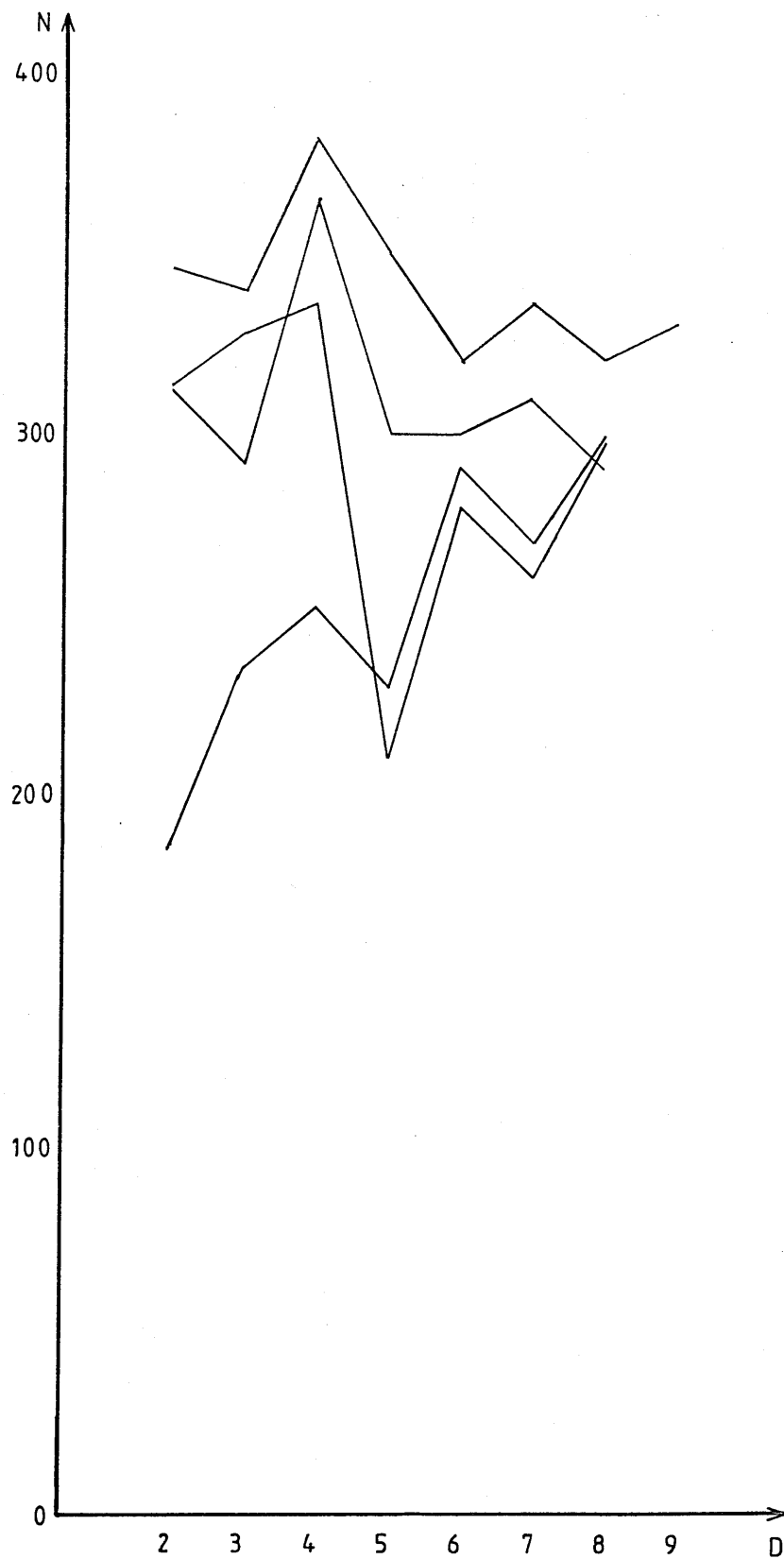
FIGURE 25



NORMAL

FIGURE 26





NORMAL

FIGURE 27

FIGURES 28, 29

Definition of measured coordinates of visual stimulus direction (S).

ON in plane of paper; OA and OS obliquely towards the reader.

Text page 66.

Figure 28

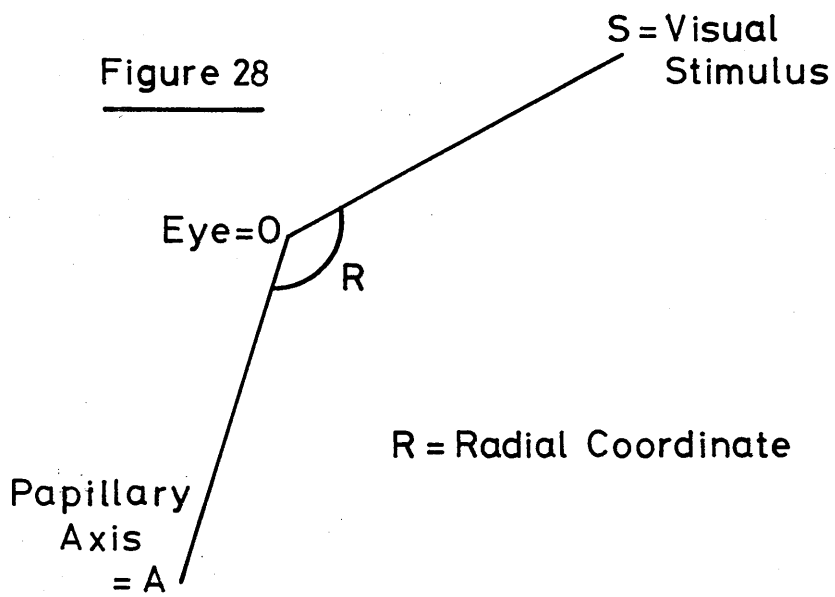


Figure 29

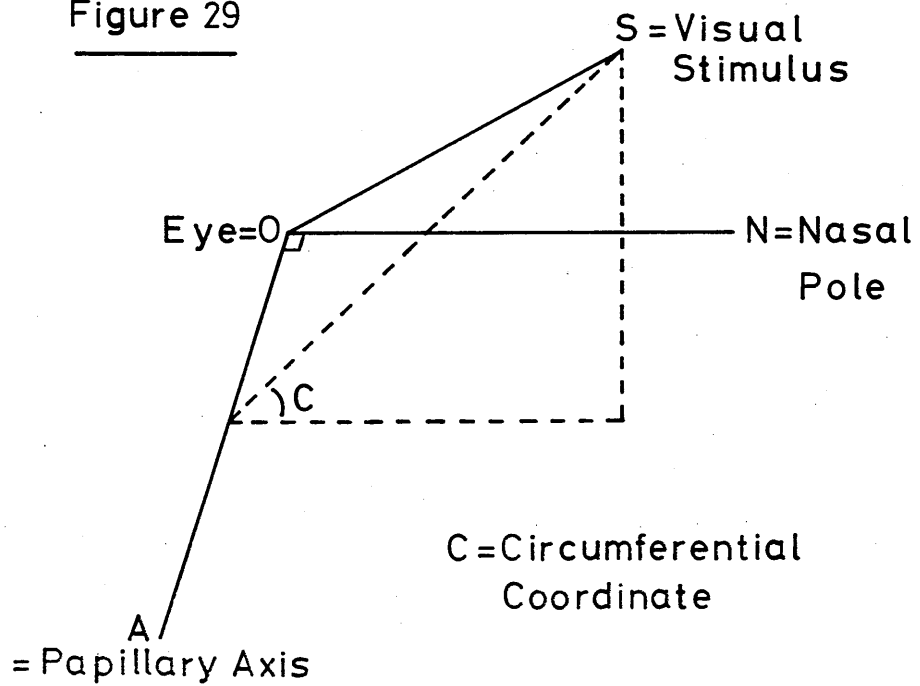


FIGURE 30

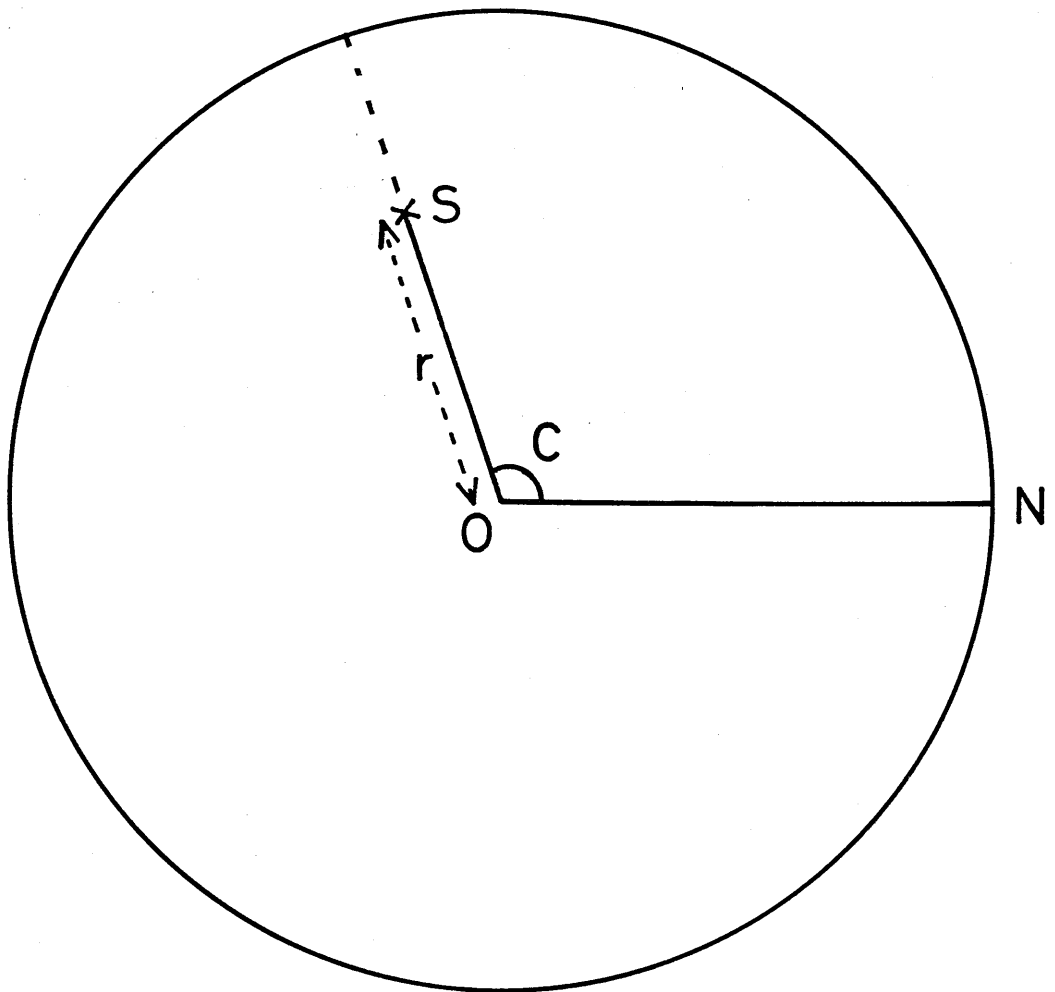
Convention by which polar coordinates of visual stimulus direction (S) are represented on a two-dimensional map.

All in plane of paper.

Text page 66.

Compare with upper diagrams of figures 50a to 78a.

Figure 30



$C$  = Circumferential  
Coordinate

$r$  = Radial Coordinate

FIGURE 31

The convention by which the curved left tectum is represented in two dimensions. Points on the true edge of the tectum are projected on to a horizontal plane at points X.

Compare with lower diagrams, figures 50a to 78a, and figures 32, 34, 36.

Text page 67.

## Horizontal Projection: Left Tectum

x = measured boundary points

• = centre of arc fitted to x's

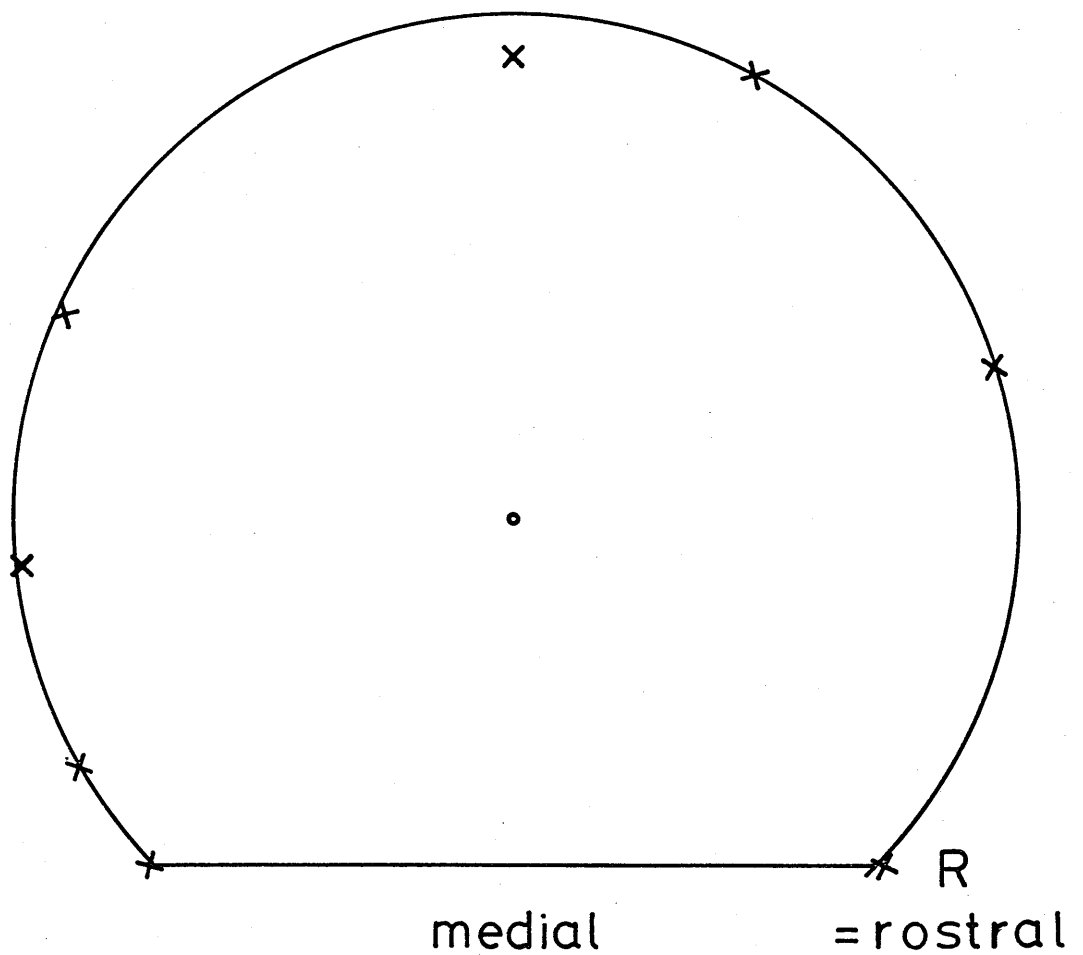


FIGURE 31

#### FIGURES 32 - 37

These figures refer to Appendix A, pages 81 - 82.

Figures 32, 34, 36: Boundary, X's and ° as in figure 31.

Alphanumeric codes (e.g. a1) show the horizontal projections of points on the surface of the tectum.

Figures 33, 35, 37:  $y$  = measured vertical coordinates of points on tectal surface.  $x$  = distance within the horizontal projection of each mapped surface locus measured from °, the projection of the true centre of the tectum.

$r$  = estimated radius of the tectum.

Most surface loci lie on or close to an arc of a circle with radius  $r$ ; therefore it is reasonable to use a spherical surface as a model of the tectum.



Horizontal Projection of Tectum 1

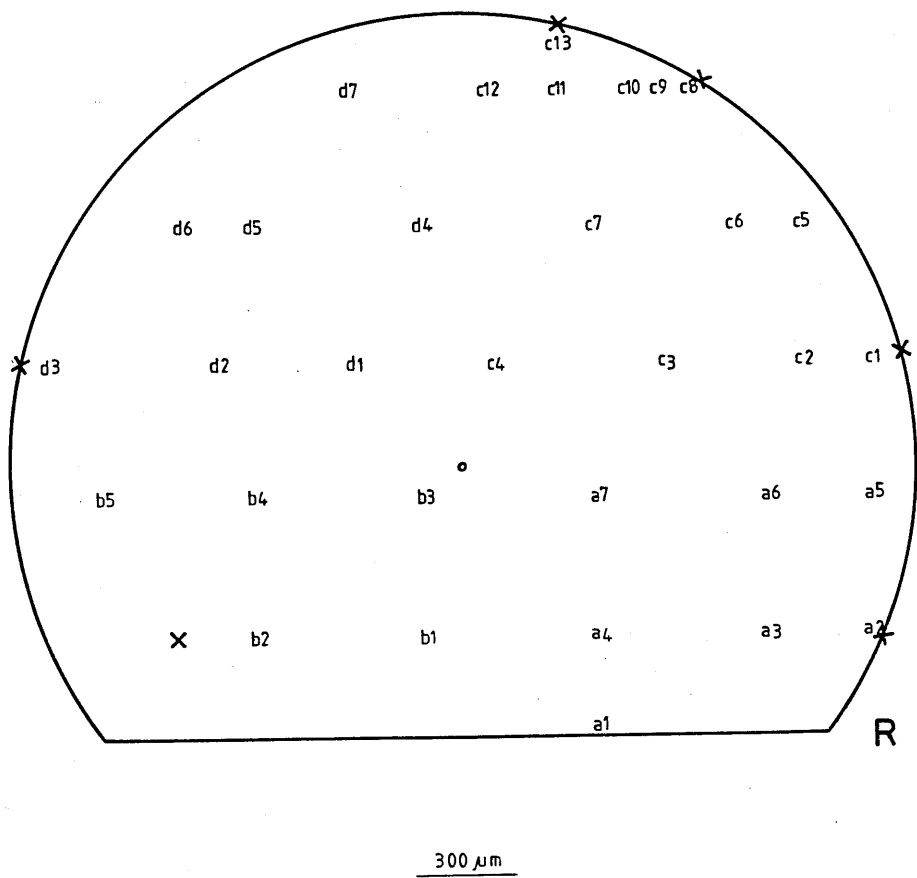
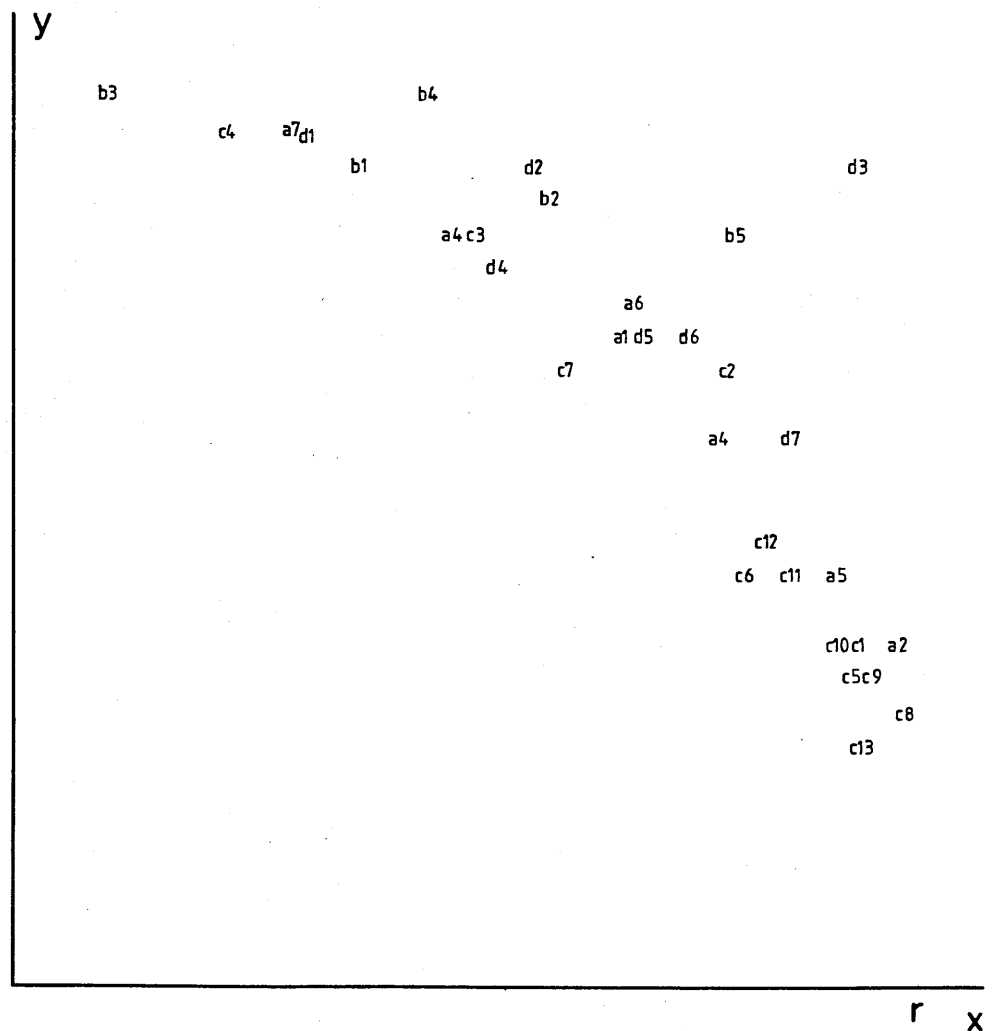


FIGURE 32

# Tectum 1



x = Horizontal Coordinate

y = Vertical Coordinate

r = Radius estimated from horizontal  
projection of perimeter

FIGURE 33

# Horizontal Projection of Tectum 2

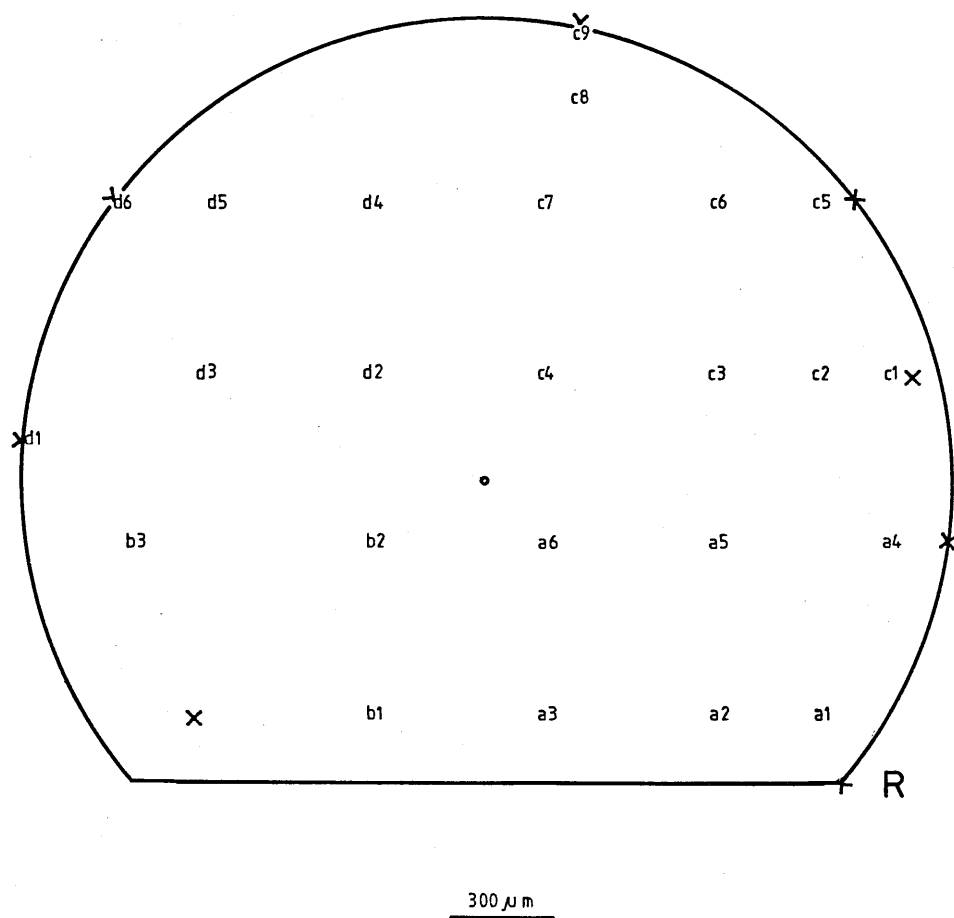


FIGURE 34

Tectum 2

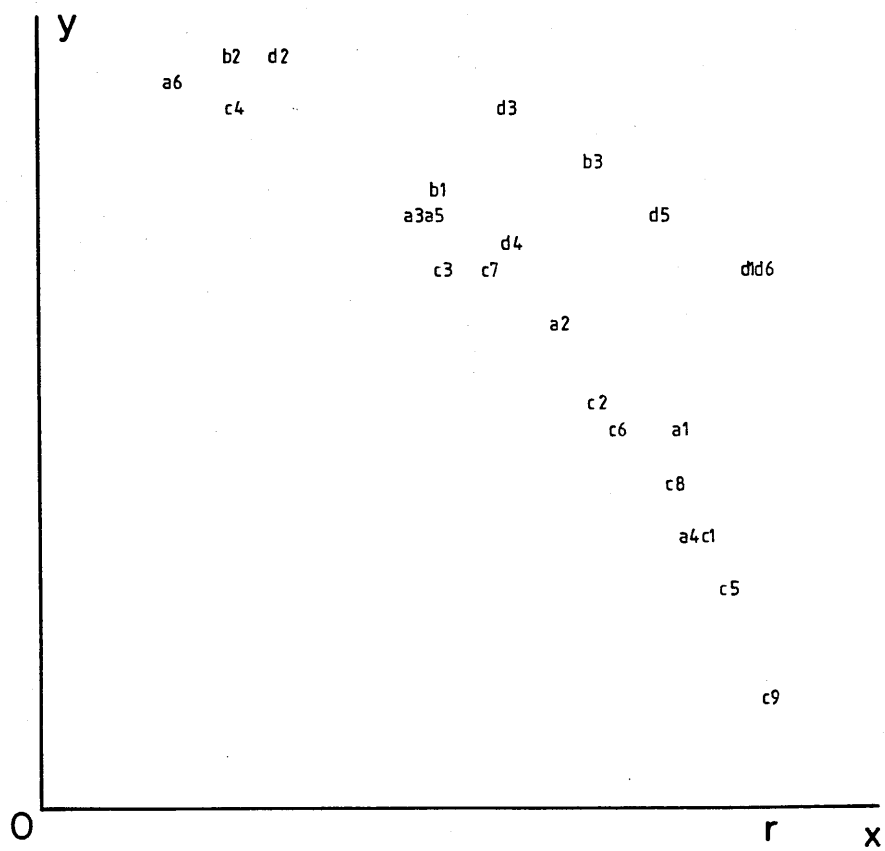


FIGURE 35

Horizontal Projection of Tectum 3

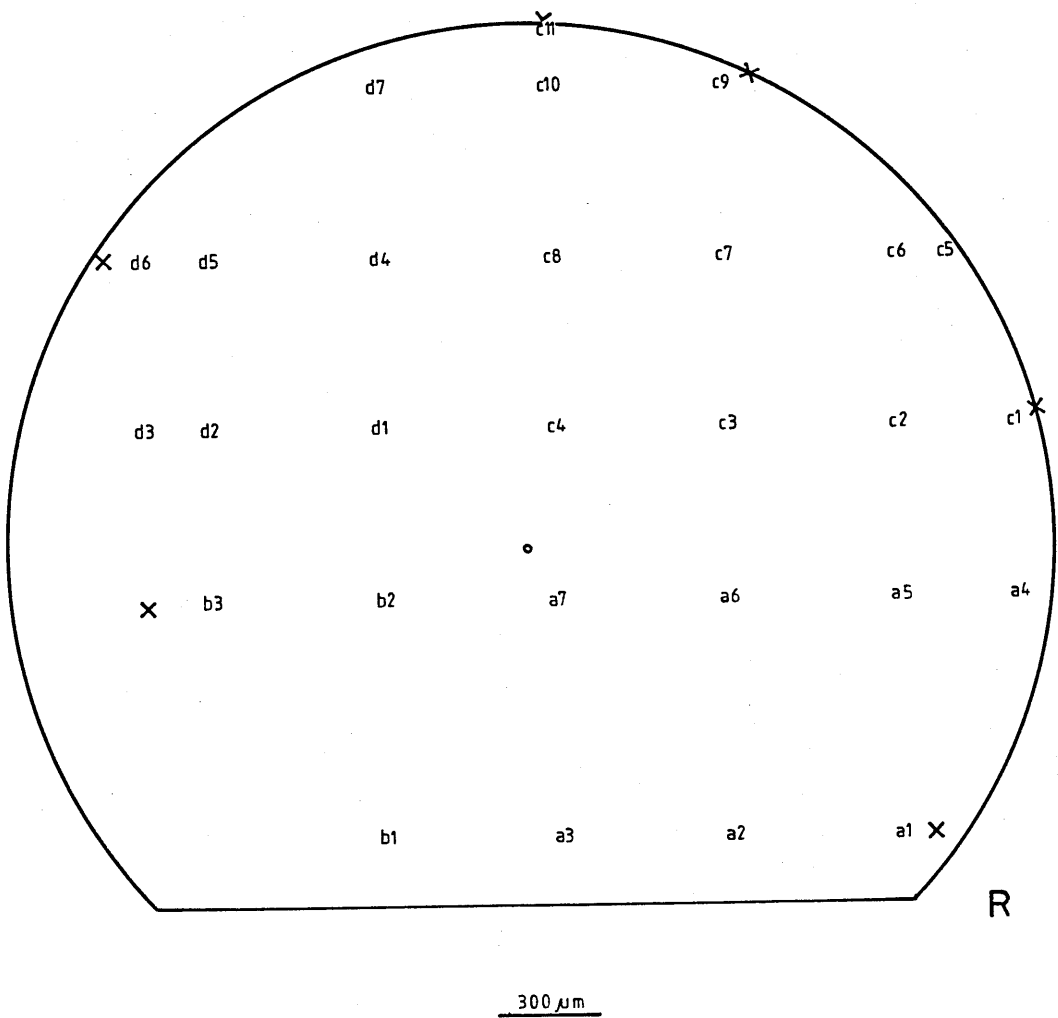


FIGURE 36

# Tectum 3

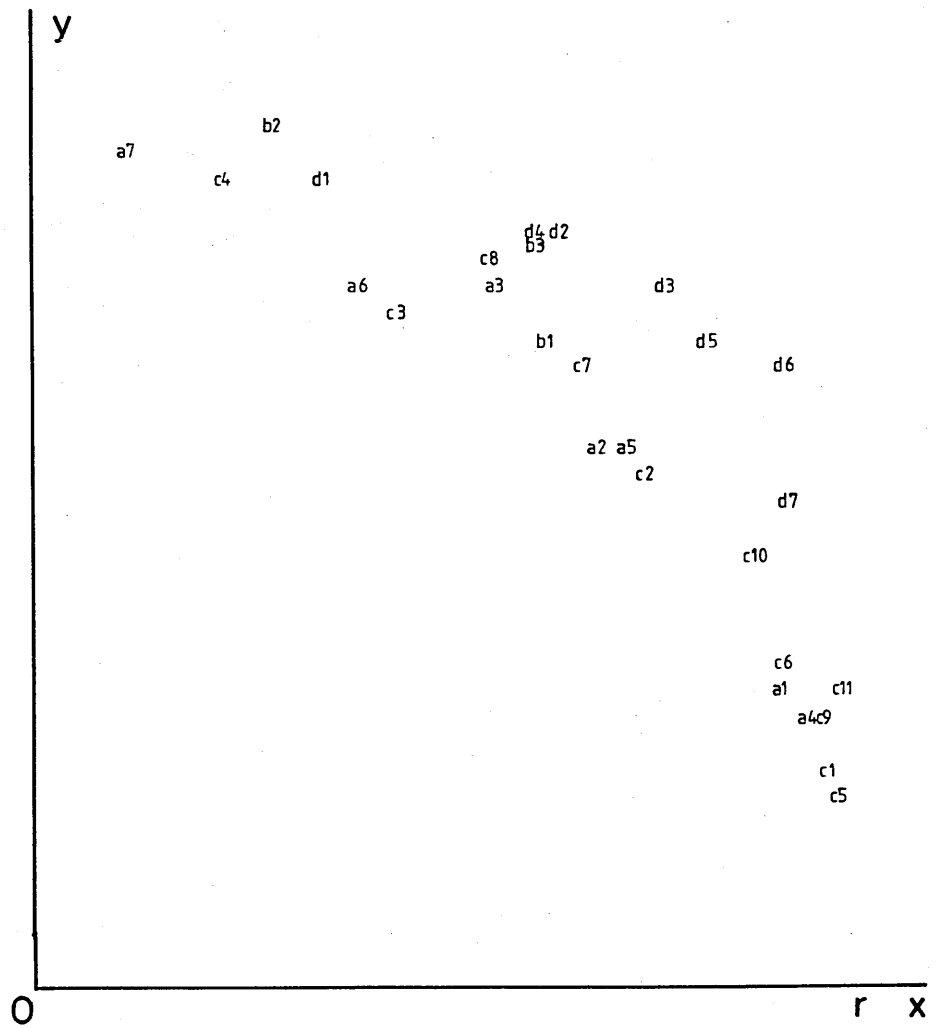


FIGURE 37

FIGURES 38 - 49

These figures refer to Appendix B, text pages 83 - 90.

Figure 38:  $O, f, g$  in plane of paper;  $h$  obliquely towards the reader.

$f, g, h$  are orthogonal.

Figure 39:  $O, f, g, h$  as in figure 38.  $G$  is on the  $g$  axis.

$OS'$  obliquely towards the reader;  $OA'$  obliquely away.

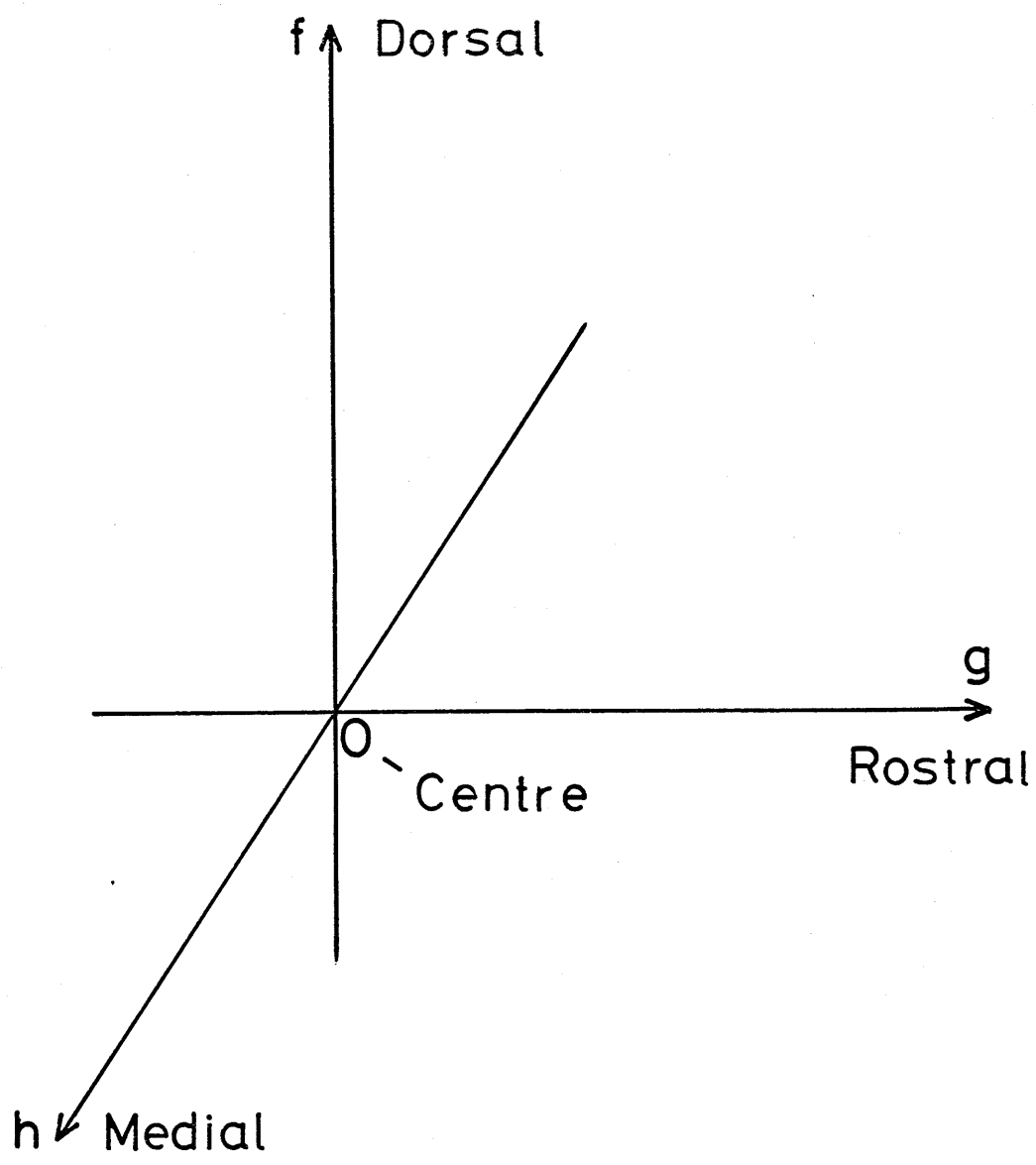
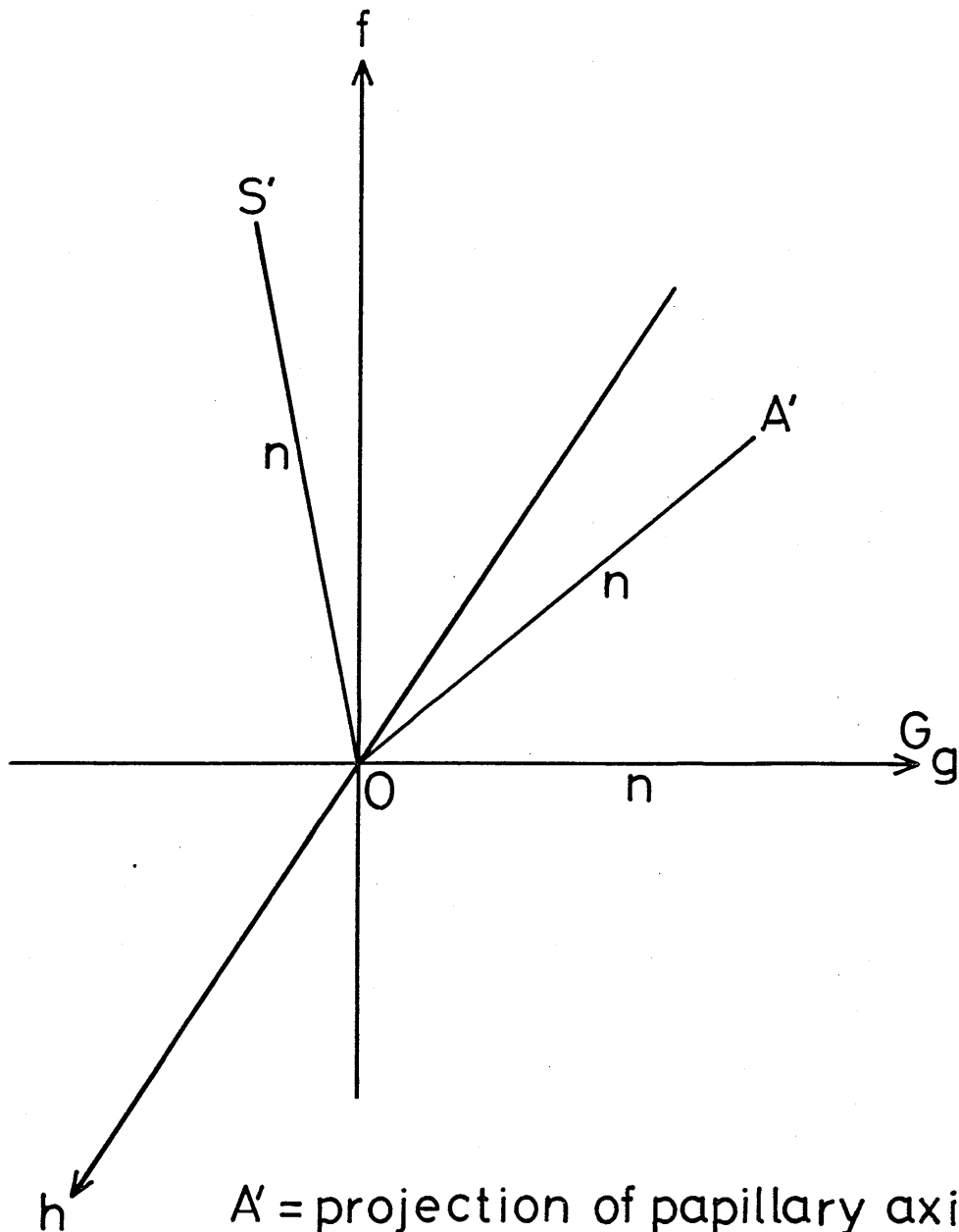


FIGURE 38



FIGURE 39



A' = projection of papillary axis A

G = rostral pole of tectum

S' = projection of visual stimulus S

n = radius of tectum

FIGURE 40

As figures 38, 39.

$(f_s, g_s, h_s)$  are coordinates of S'

$(f_a, g_a, h_a)$  are coordinates of A'

$r$  = radial coordinate of S', calculated from these coordinates and  
from the estimated radius of the tectum ( $n$ ). See page 84.

Figure 40

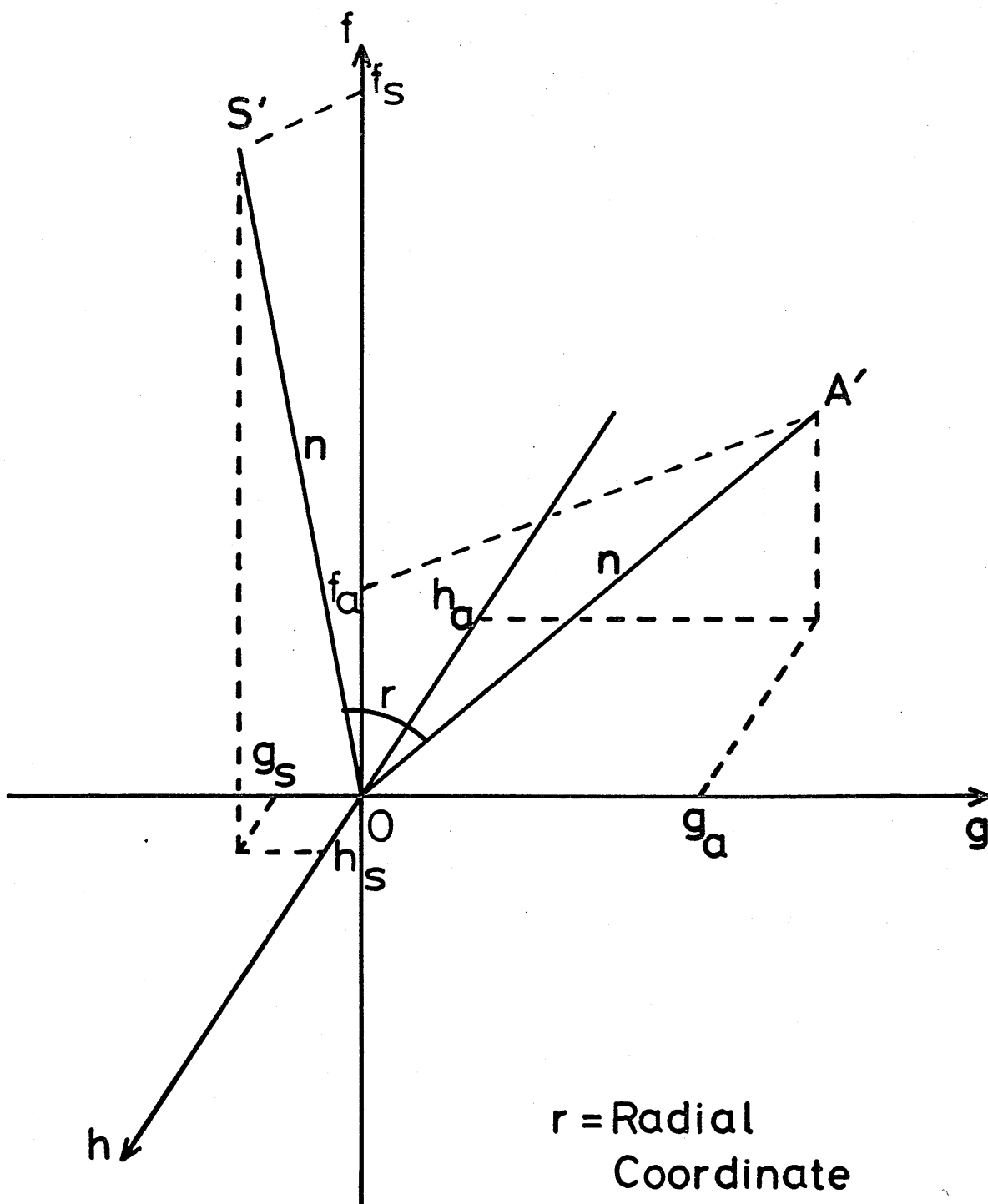


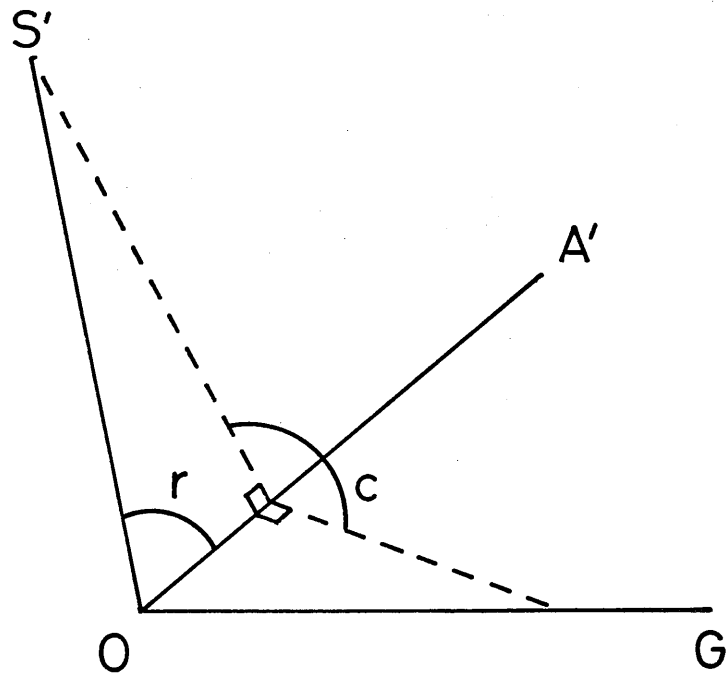
FIGURE 41

O, G, A', S', r as in figures 38 - 40.

OS' obliquely towards the reader, OA' obliquely away, OG in plane of paper.

C = angle between plane OA'S' and plane A'OG = circumferential coordinate of S'.

FIGURE 41



$r$  = Radial coordinate of  $S'$

$c$  = Circumferential  
coordinate of  $S'$

FIGURE 42

O, G, A', c as in figure 41.

x axis = OA' produced

y axis = co-planar with A'OG, and obliquely towards the reader.

z axis = obliquely towards the reader.

x, y, z axes are mutually perpendicular.

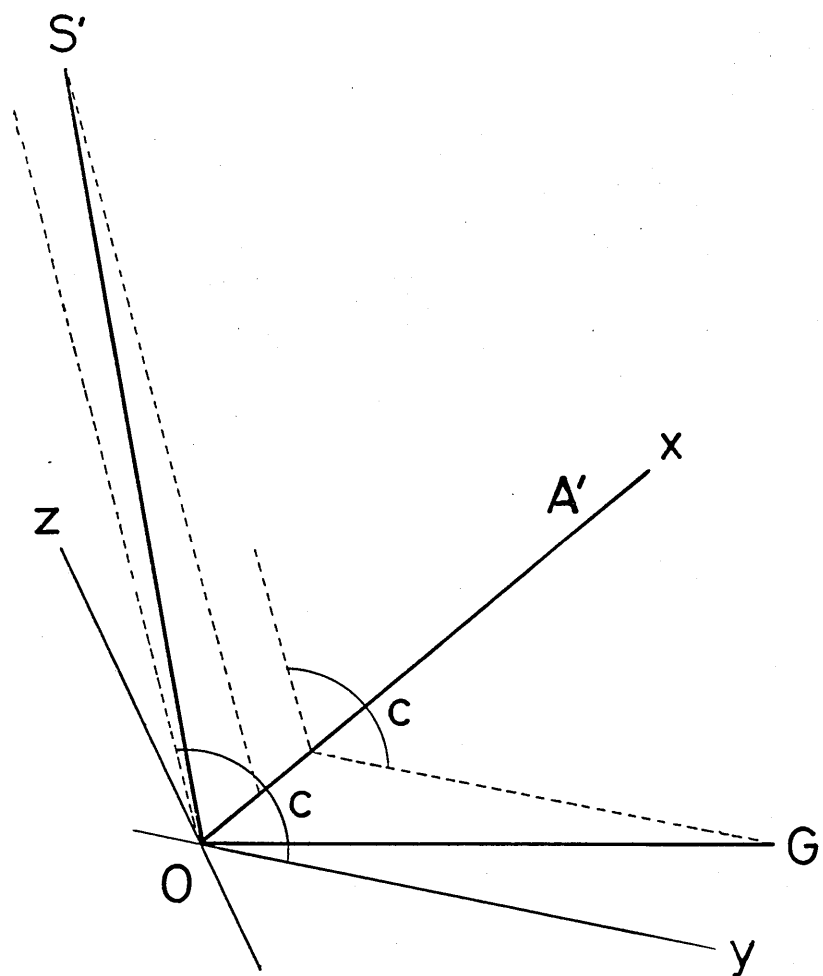


FIGURE 42

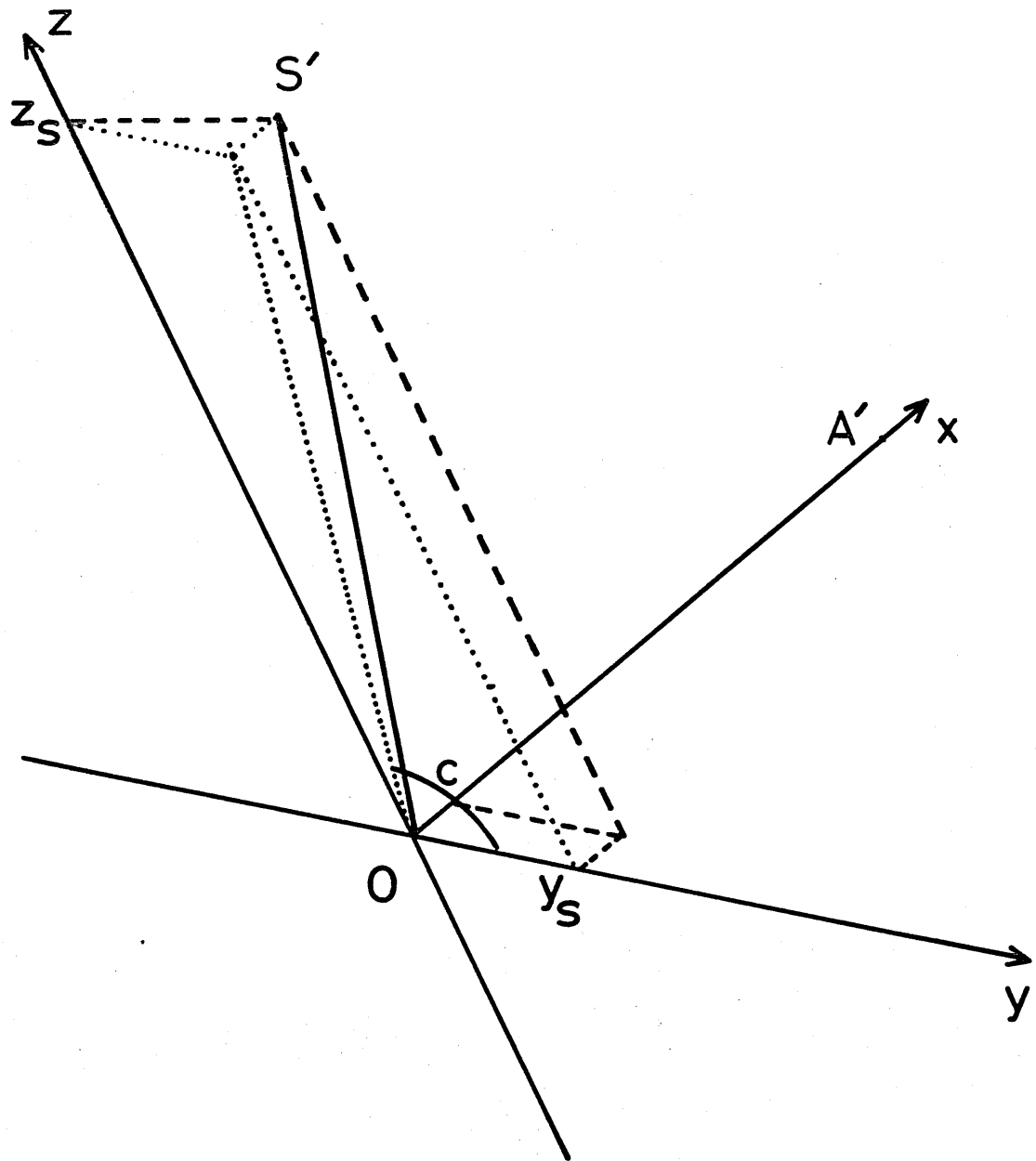
FIGURE 43

As figure 42.

$y_s, z_s$  are coordinates of  $S'$ .  $x_s$  is not shown.



Figure 43



$c$  = Circumferential Coordinate

FIGURE 44

As figure 42

$x_g, y_g$  = coordinates of G. ( $z_g$  = zero)

OP = vector along x axis, magnitude  $x_g$

OQ = vector along y axis, magnitude  $y_g$



FIGURE 45

$\underline{i}$ ,  $\underline{j}$ ,  $\underline{k}$  are unit vectors in  $f$ ,  $g$ ,  $h$  axes.

$\underline{i}$ ,  $\underline{j}$  are in the plane of the paper;  $\underline{k}$  is obliquely away.

FIGURE 46

$\underline{OA}$  = vector in  $x$  axis, obliquely away from the reader.

$\underline{OQ}$  = vector in  $y$  axis, obliquely towards the reader.

$\underline{OZ}$  = vector in  $z$  axis, obliquely towards the reader.

FIGURE 45

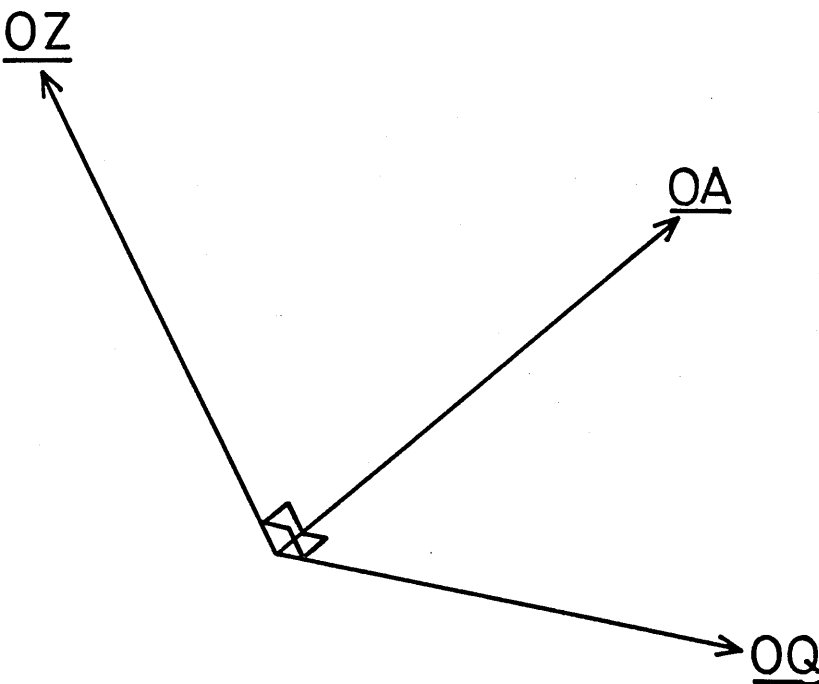
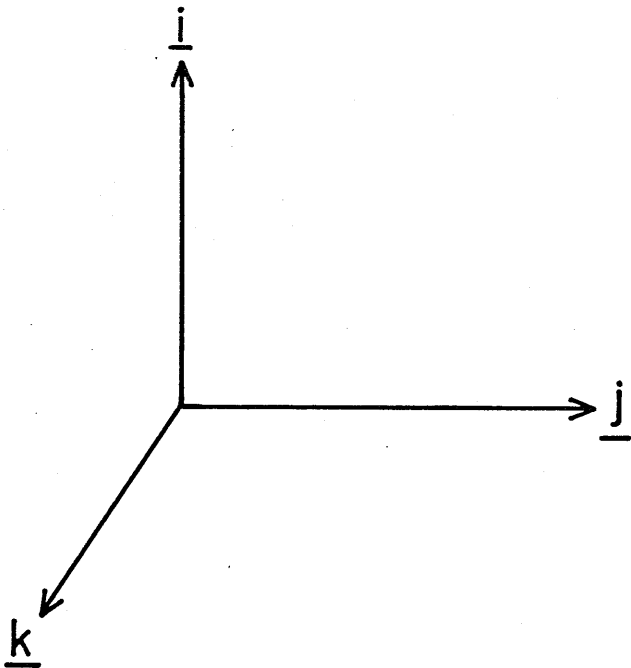
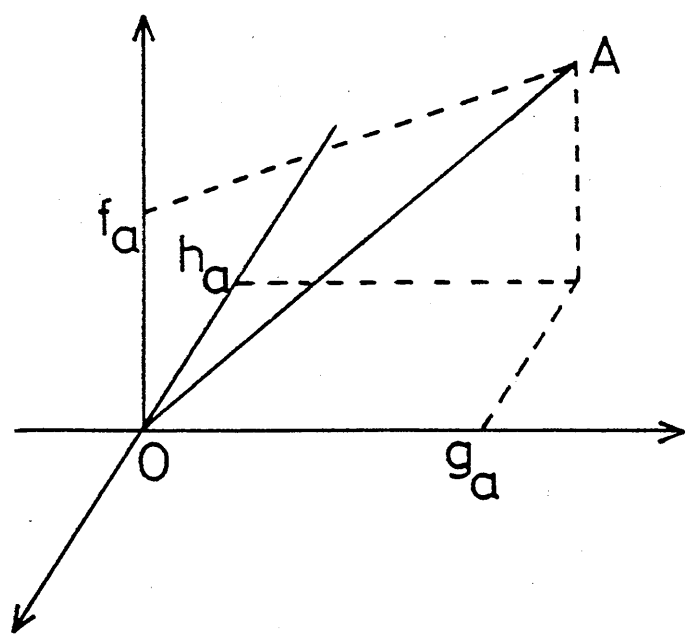


FIGURE 46

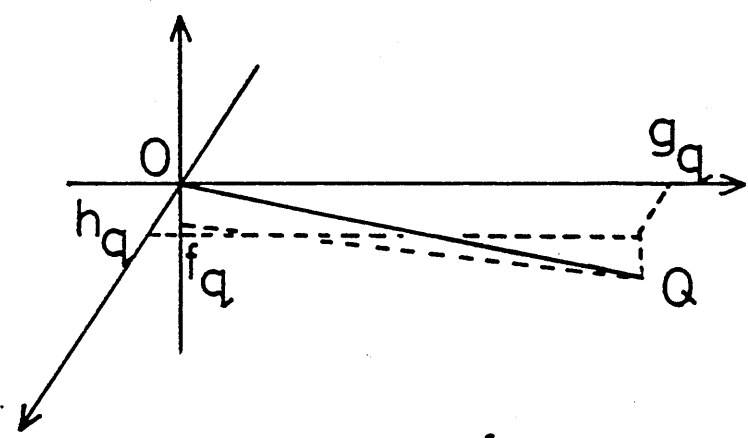
FIGURES 47, 48, 49

These figures illustrate the  $f$ ,  $g$ ,  $h$  coordinates of the vectors shown in figure 46.

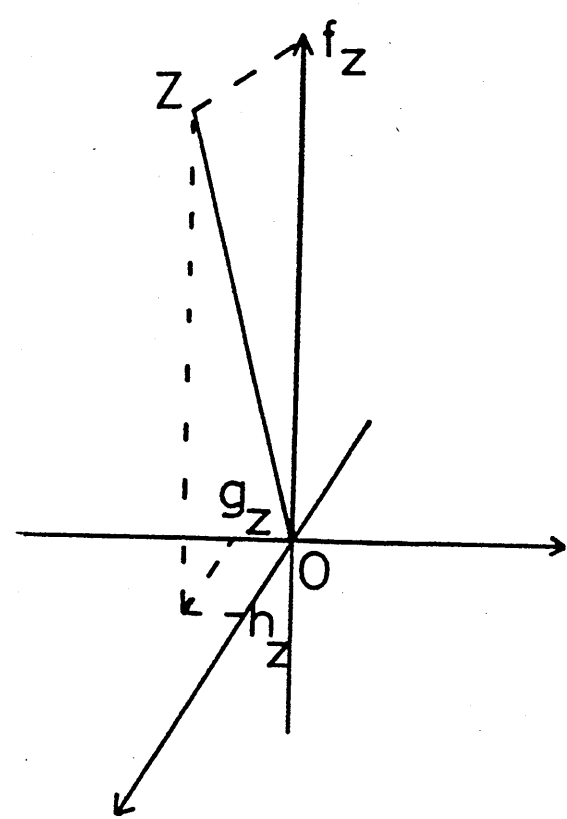
FIGURES



47



48



49

FIGURES 50 - 78

Figures "a", upper: two- dimensional representation of the visual field, depicted as explained on text page 65.

See also figure 30.

Figures "a", lower: two- dimensional representation of the tectum (horizontal projection). Text page 67, and figure 31.

Figures "a" include reference to the code-name of the fish represented in that and the succeeding figures.

Figures "b": Circumferential coordinates of visual stimuli plotted against circumferential coordinates of corresponding tectal loci. Text pages 71 - 72.

Figures "c": Radial coordinates of visual stimuli plotted against radial coordinates of corresponding tectal loci. Text pages 71 - 72.

Figures "d" usually show standard residuals plotted against visual field coordinates for the radial dimension. See text page 73.

Exceptions and additional figures are discussed on the pages indicated below each.

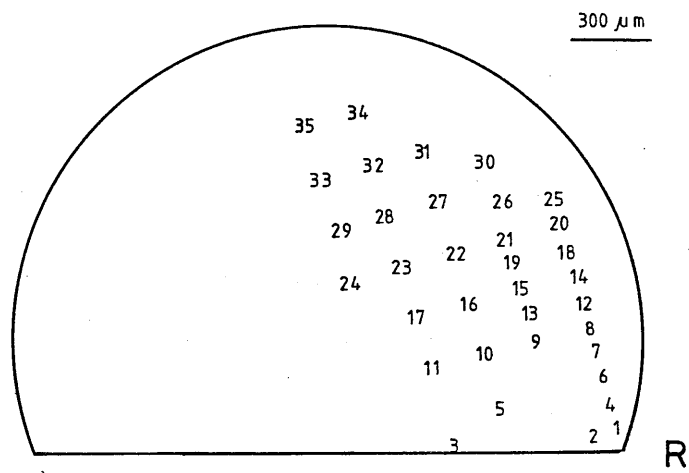
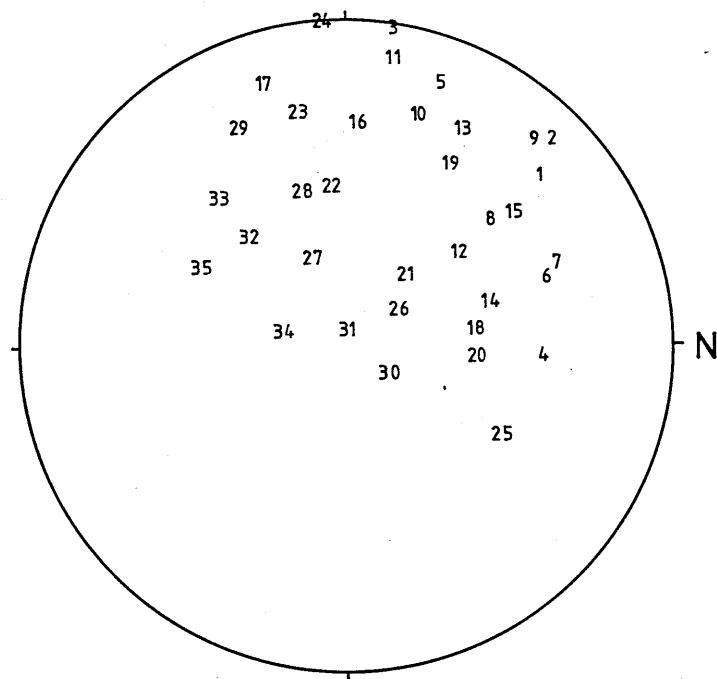
Figures 50 - 55: normal retinotectal projections. Pages 98 - 106.

Figures 56 - 63: uninterrupted regeneration. Pages 107 - 115.

Figures 64 - 69: interrupted regeneration, group A. Pp. 116 - 120

Figures 70 - 78: interrupted regeneration, group B. Pp. 121 - 129





N 1

FIGURE 50 a

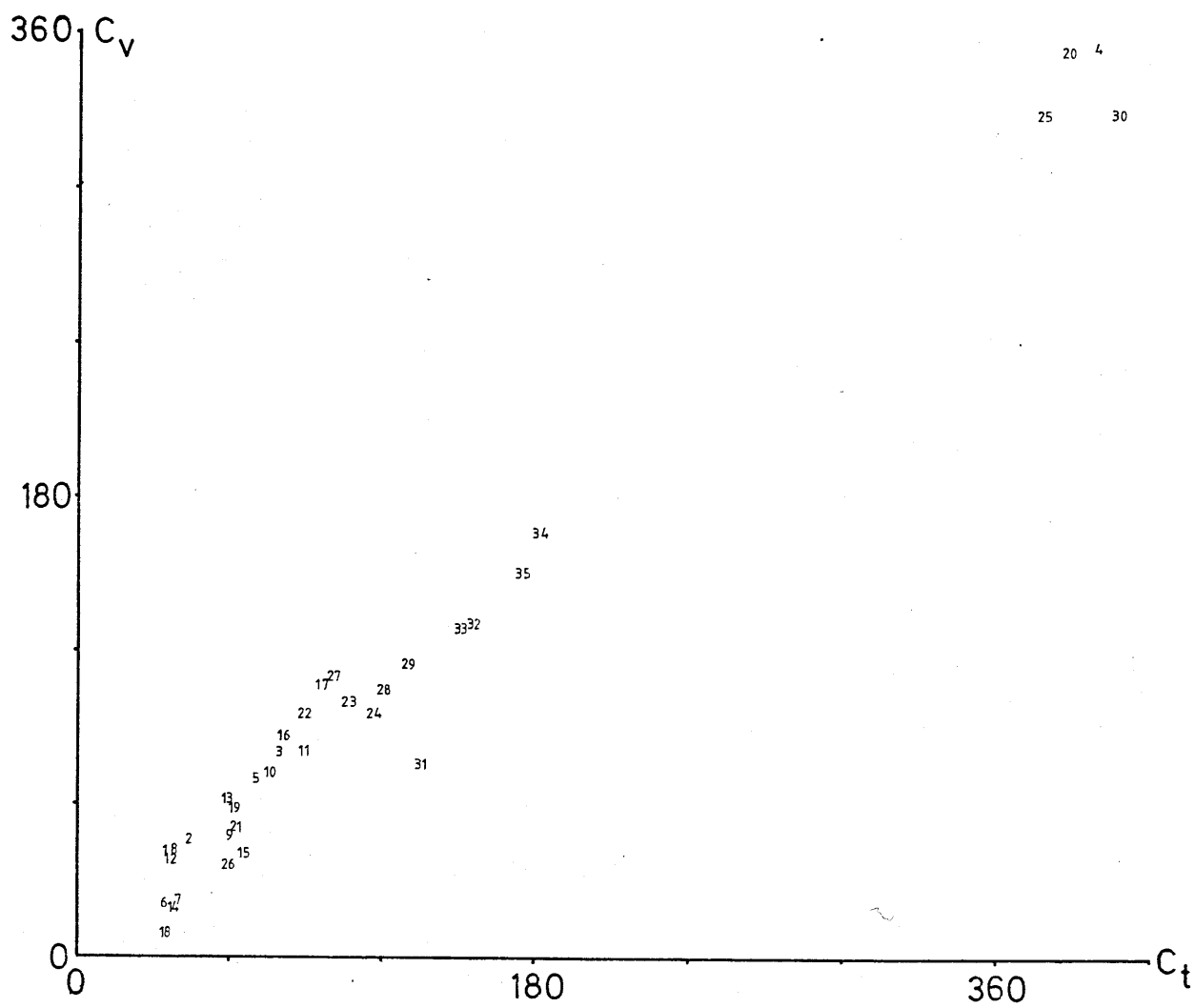


FIGURE 50 b

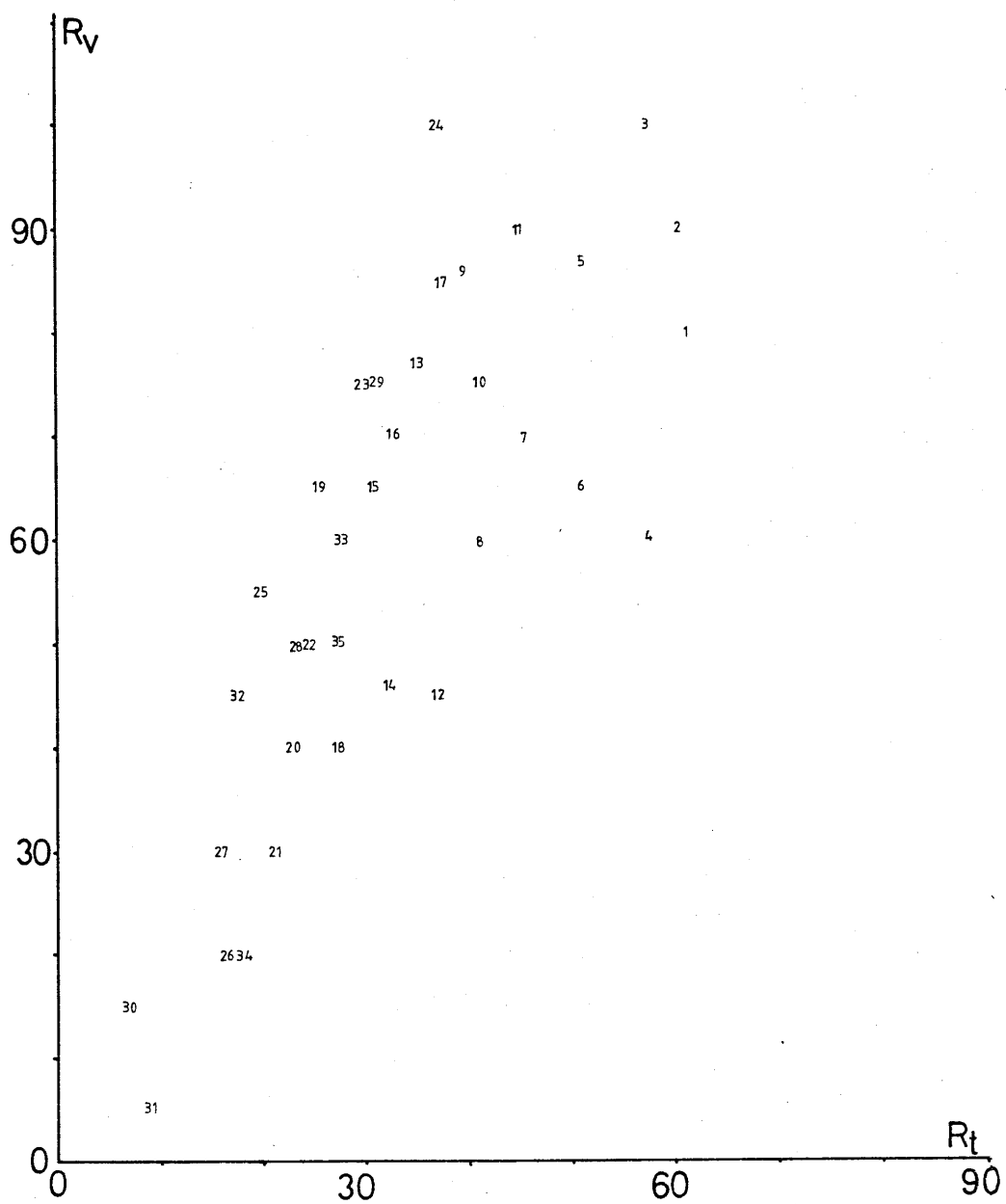


FIGURE 50c

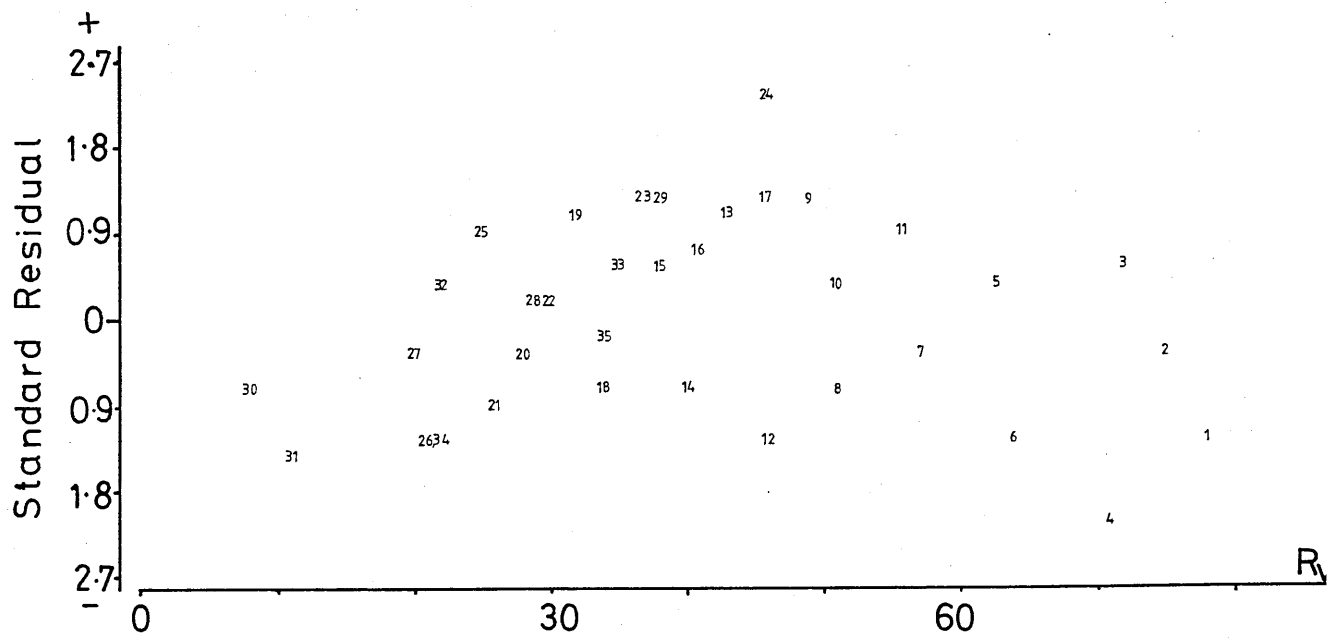


FIGURE 50d

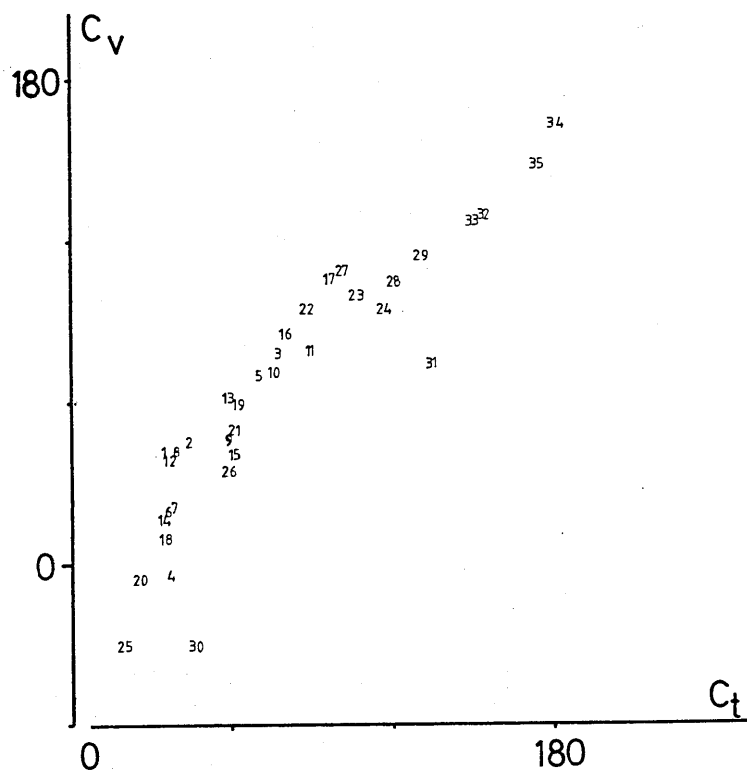
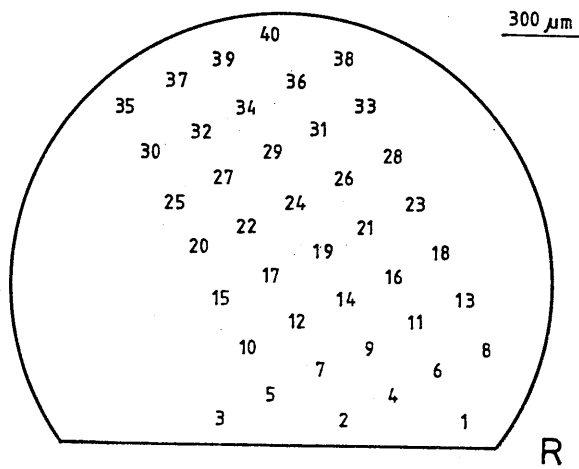
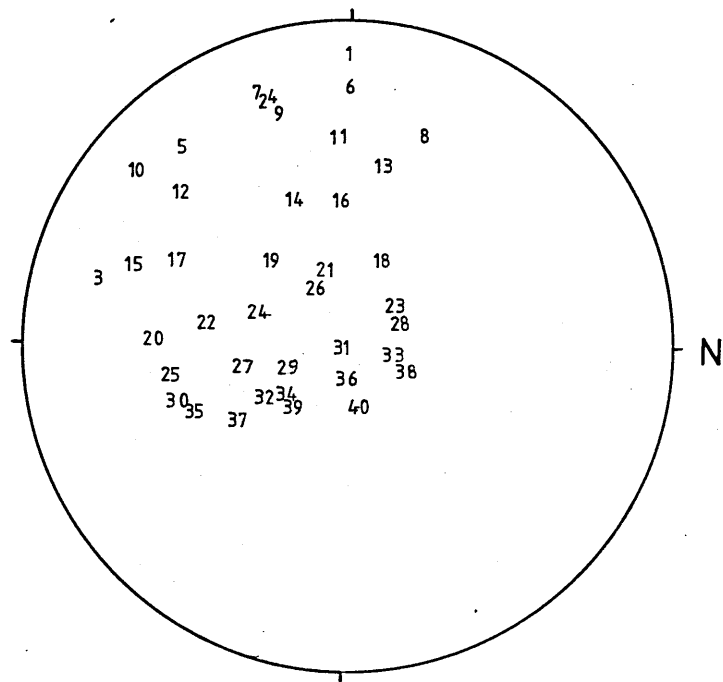


FIGURE 50 e



N 2

FIGURE 51a

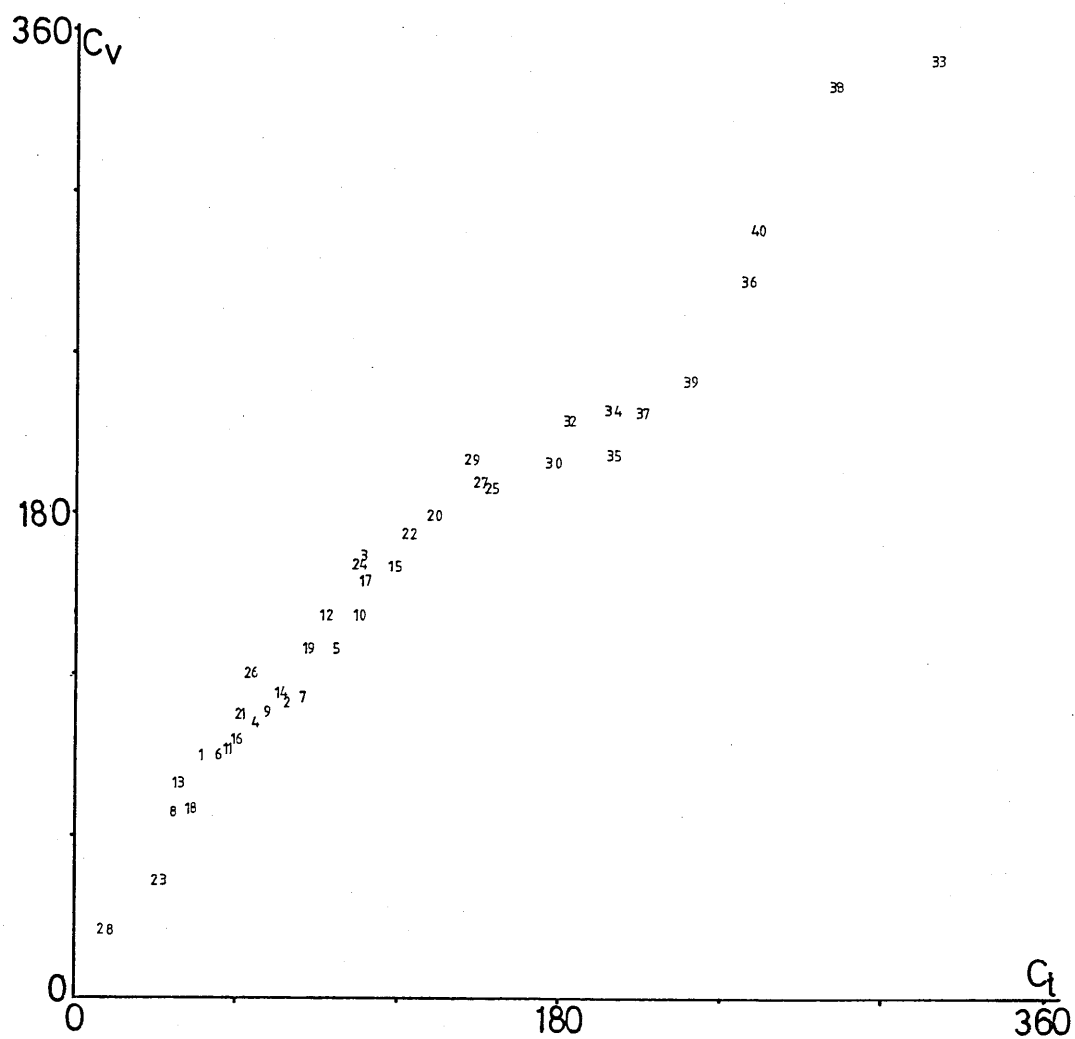


FIGURE 51 b

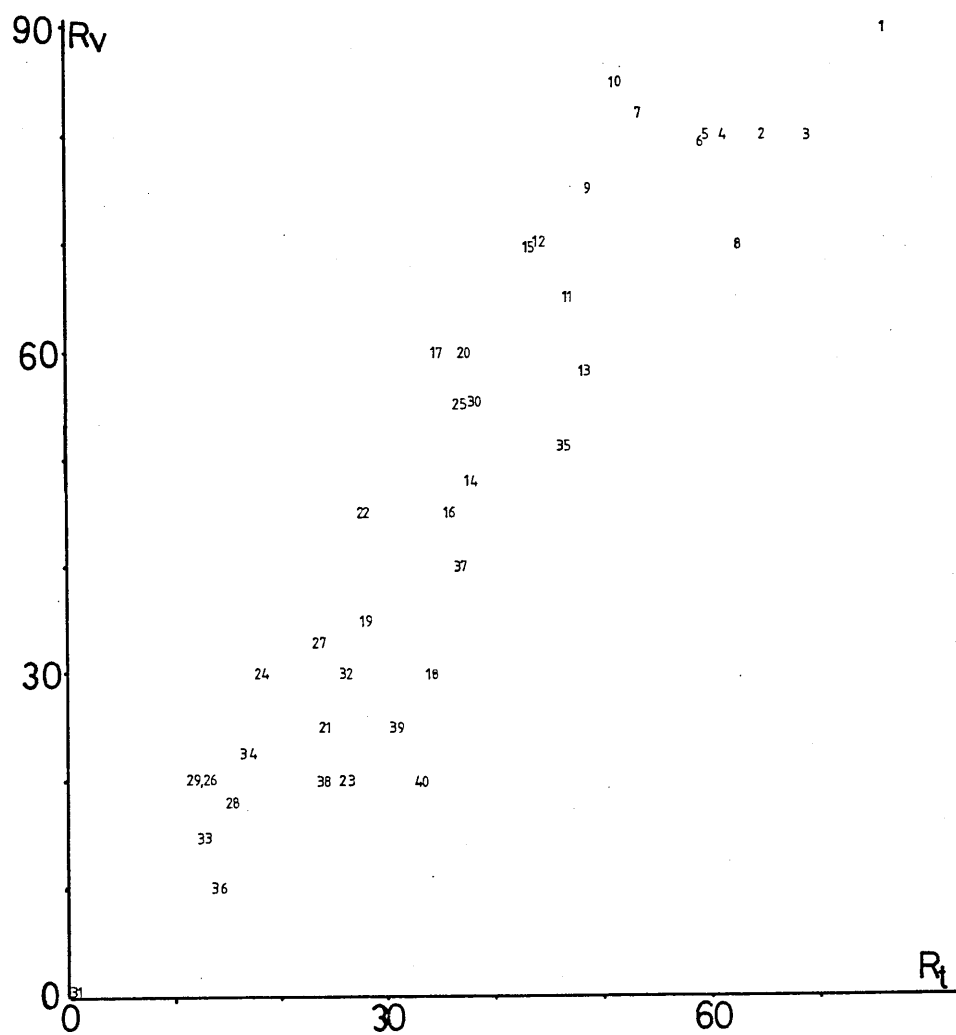
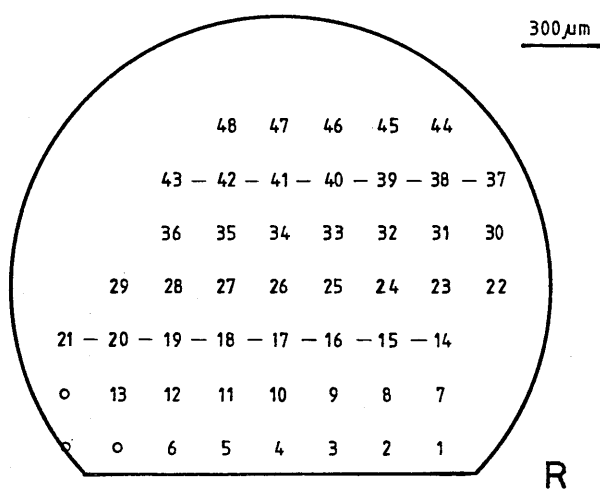
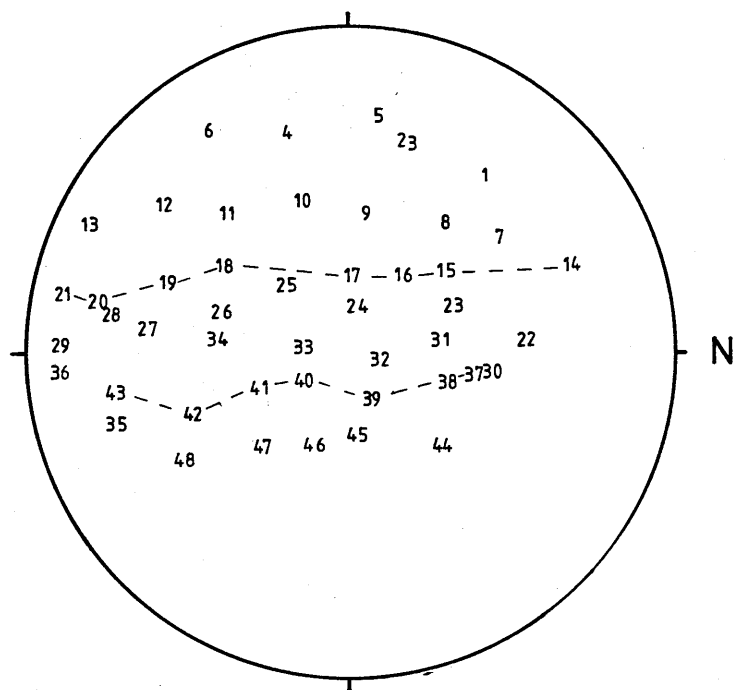


FIGURE 51c





N 3

FIGURE 52a

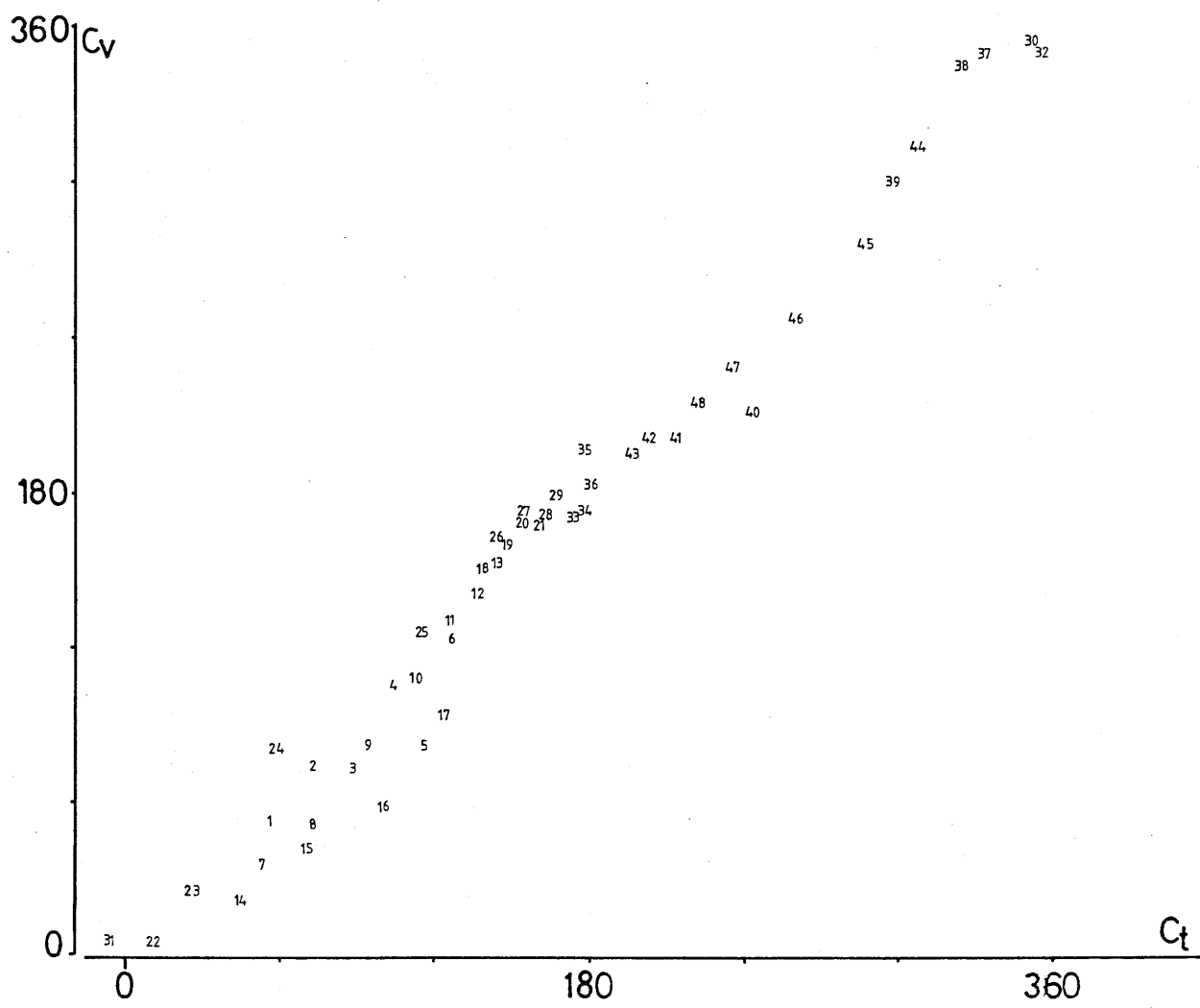


FIGURE 52 b

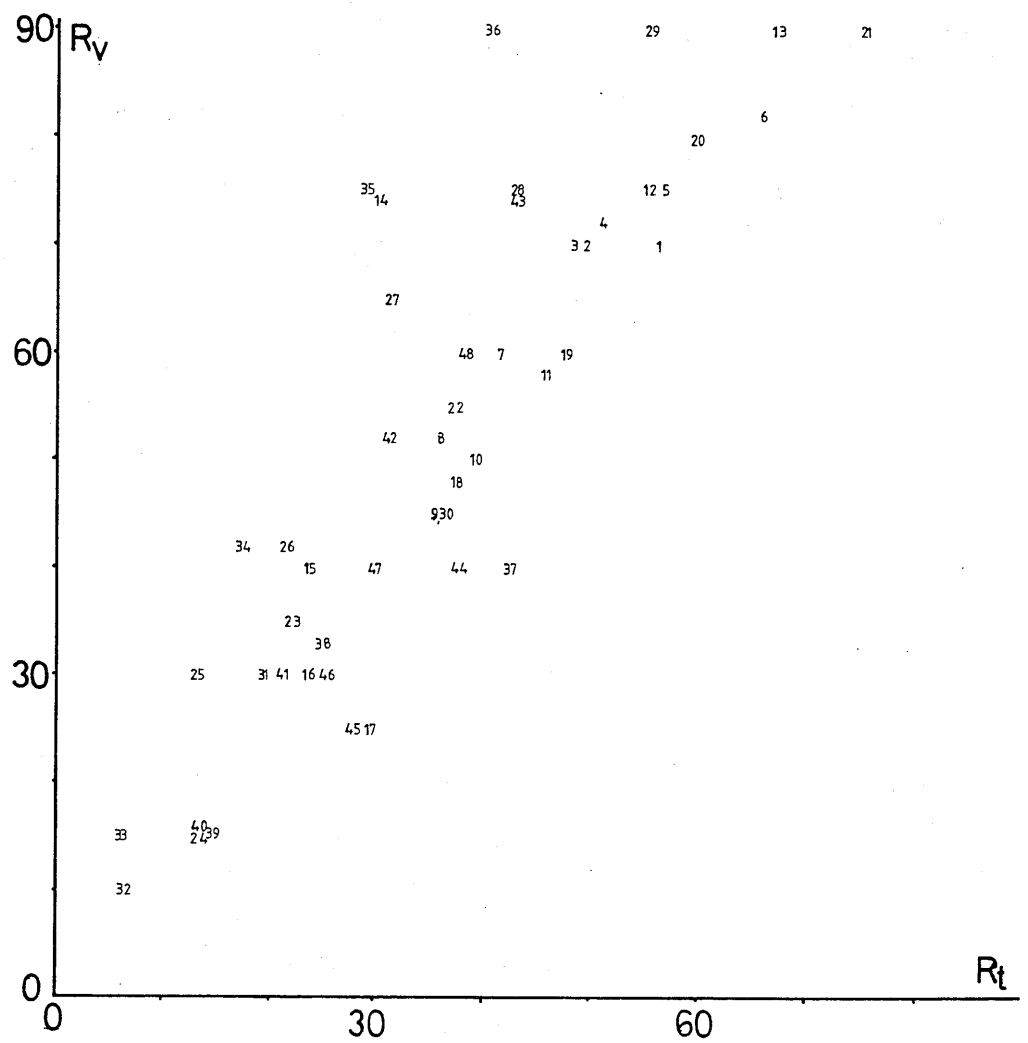
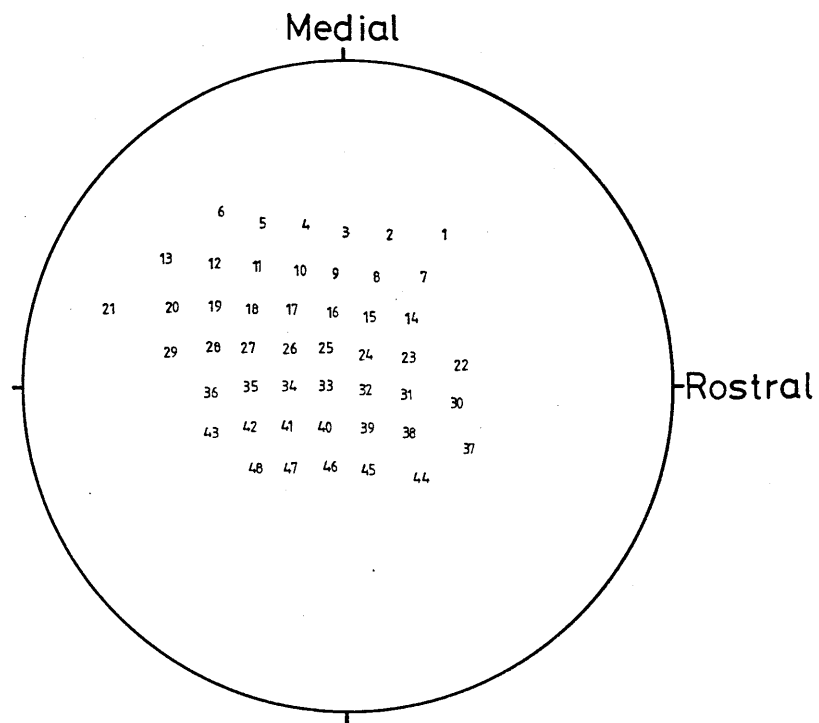
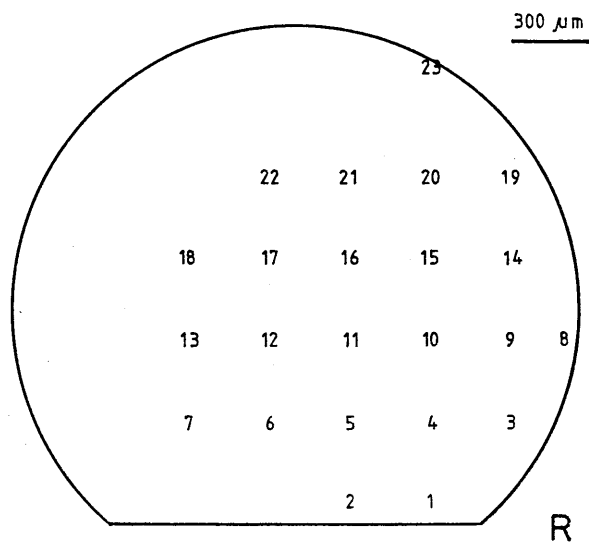
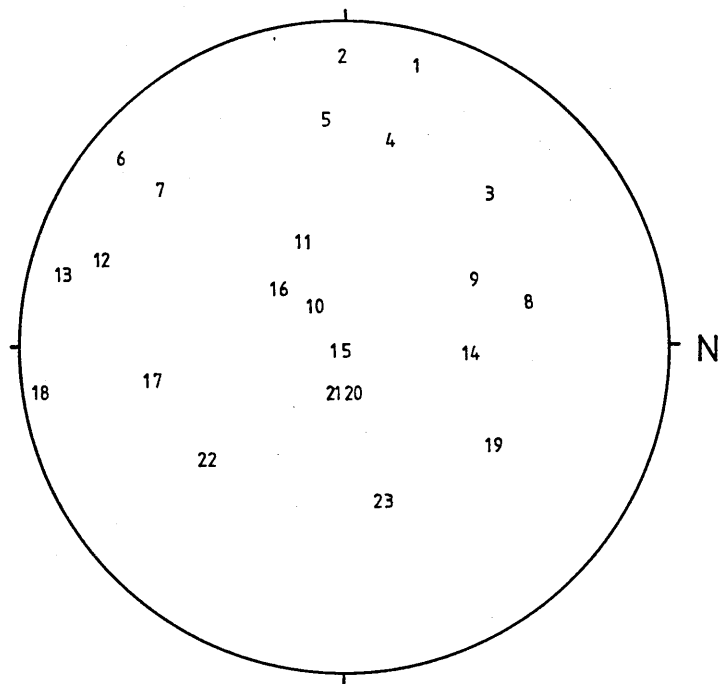


FIGURE 52 c



Recording sites plotted in polar coordinates  
computed using spherical model tectum

FIGURE 52d



N 4

FIGURE 53a

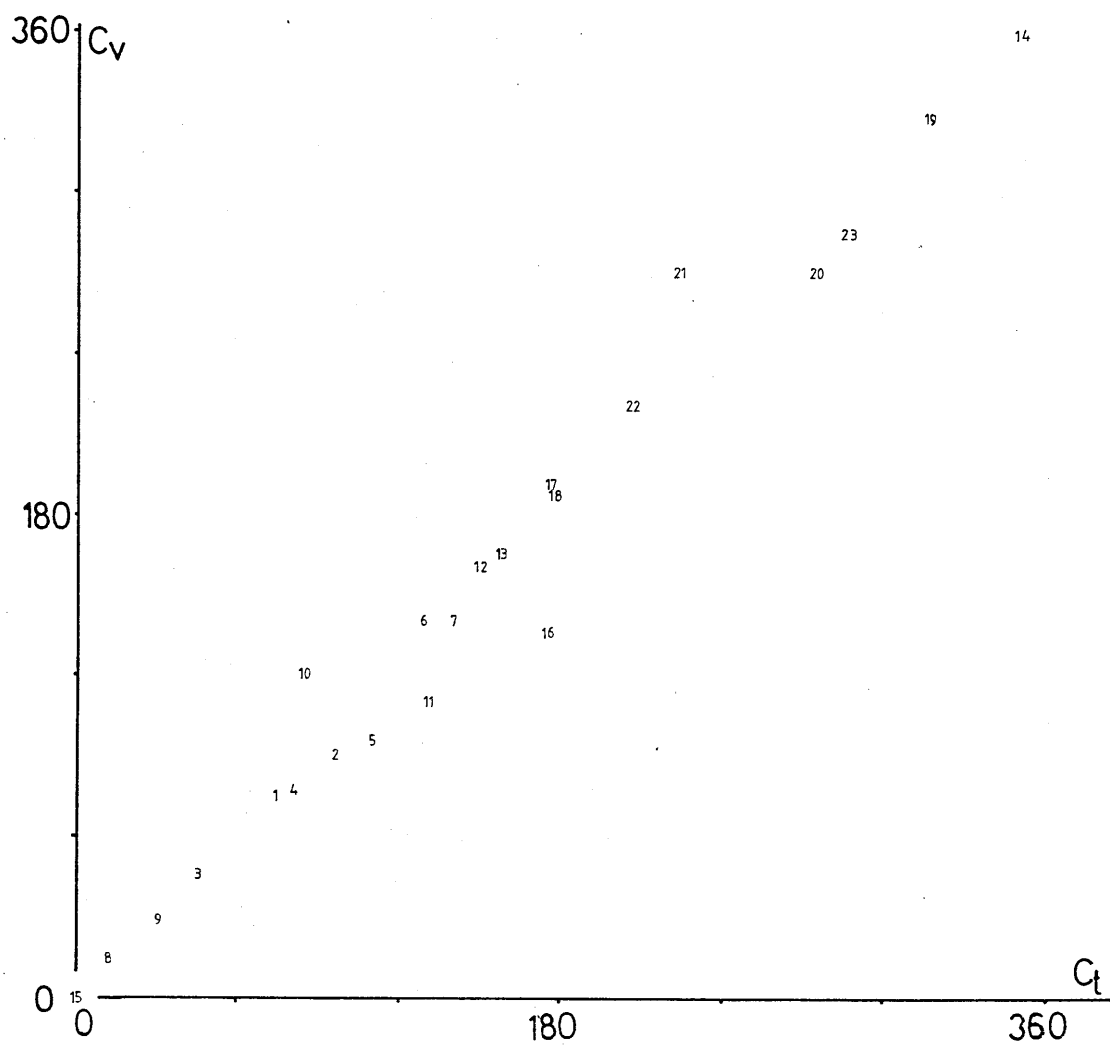


FIGURE 53 b

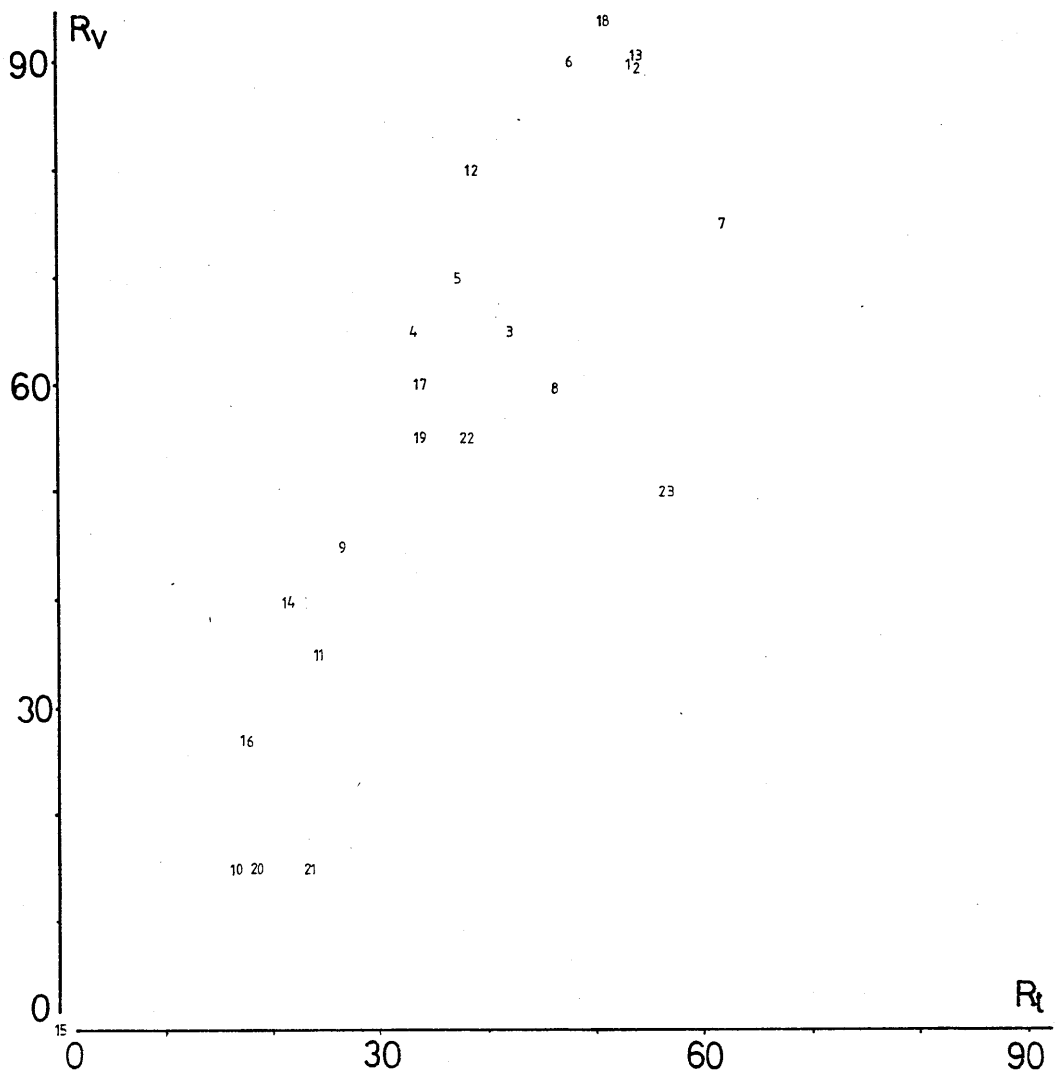
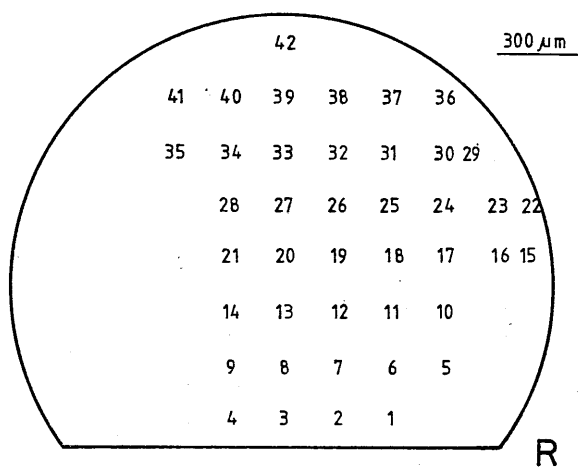
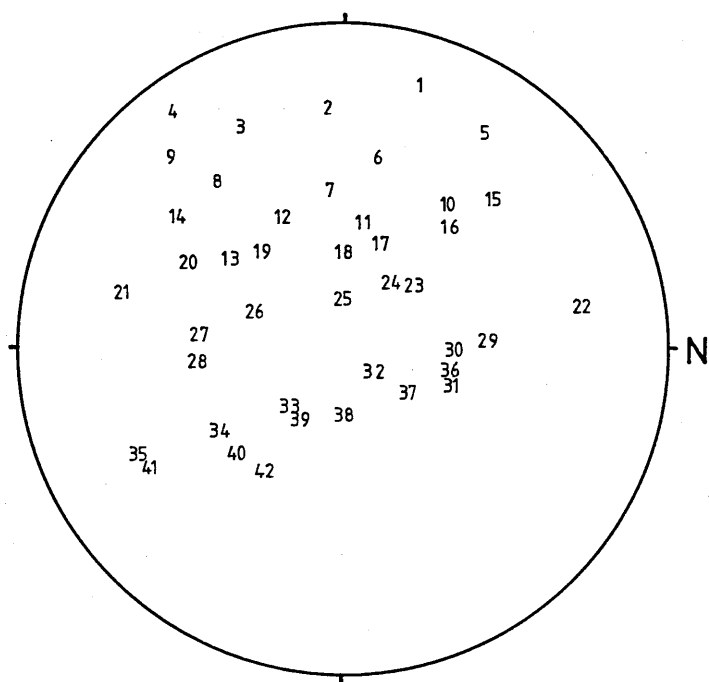


FIGURE 53 c



N 5

FIGURE 54a



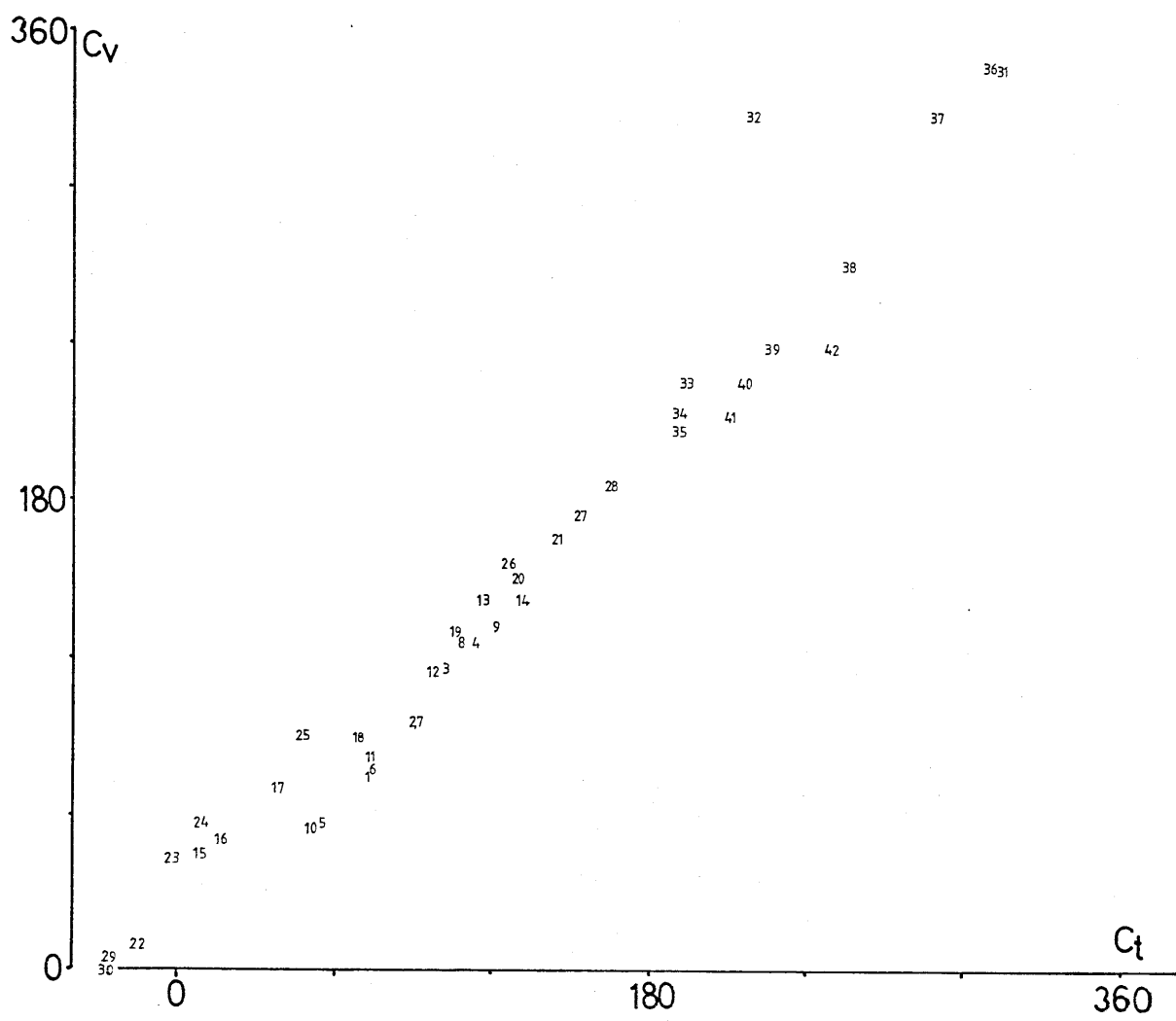


FIGURE 54b

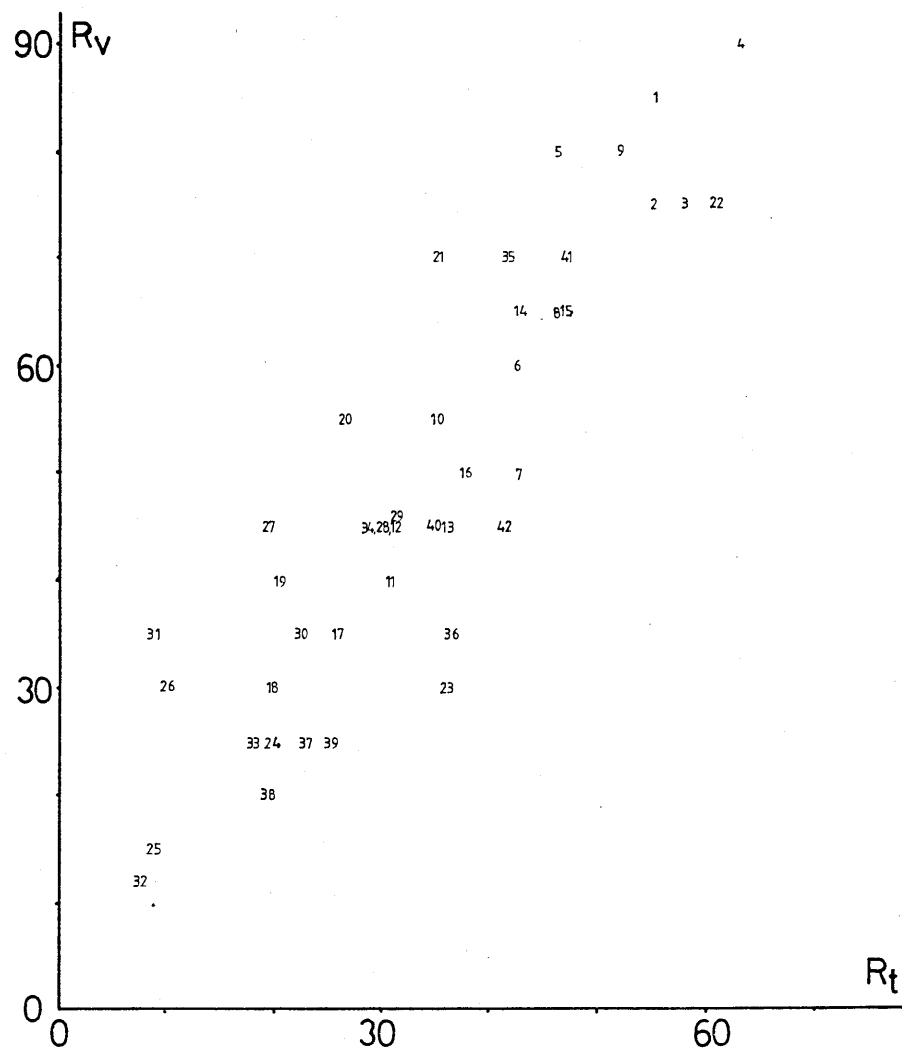


FIGURE 54c

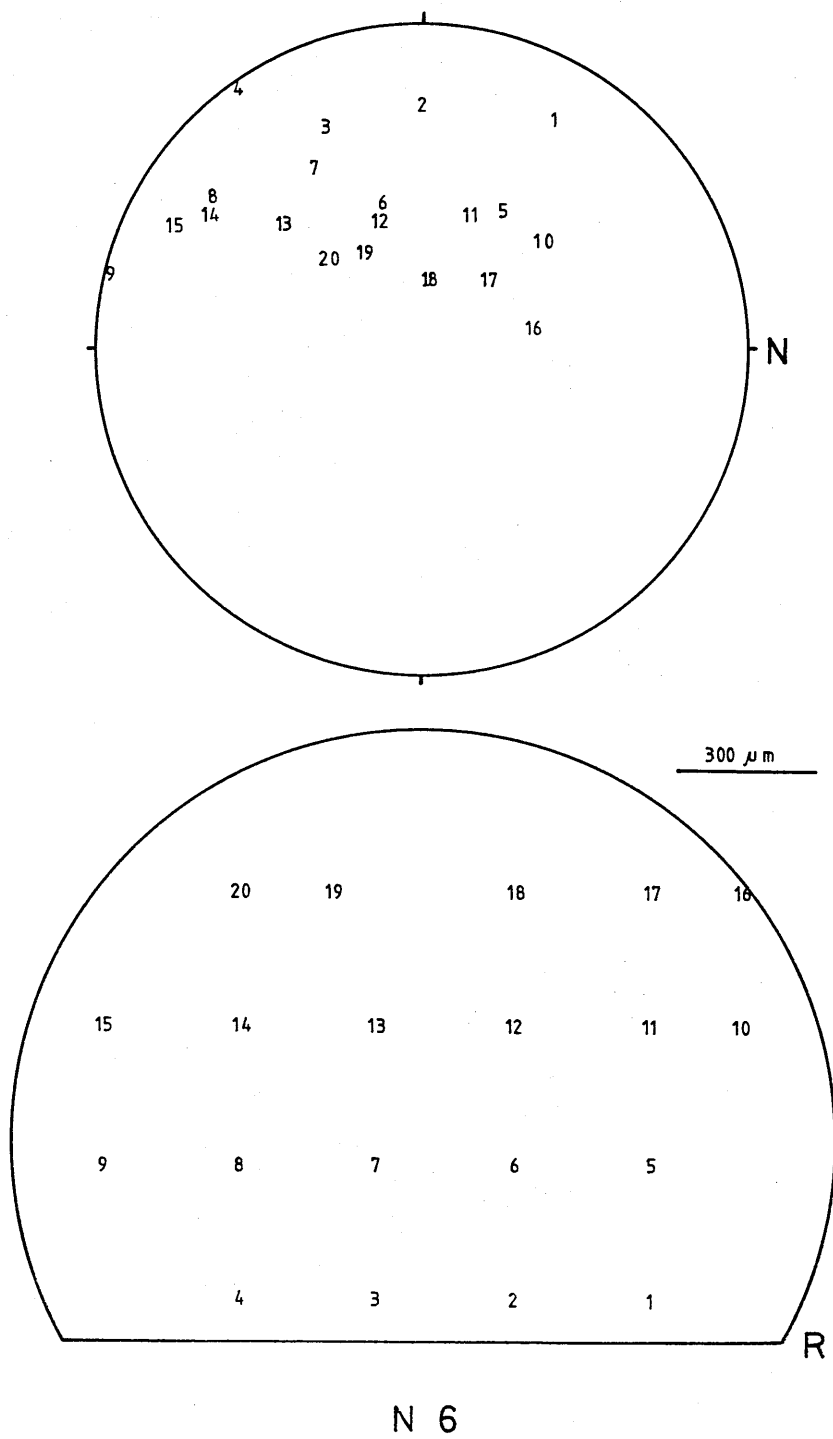


FIGURE 55a

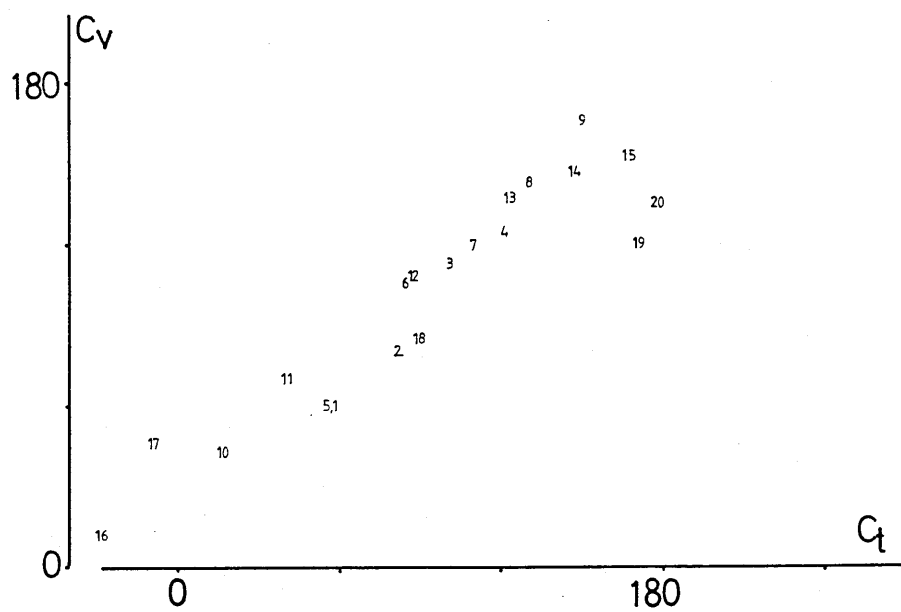


FIGURE 55 b

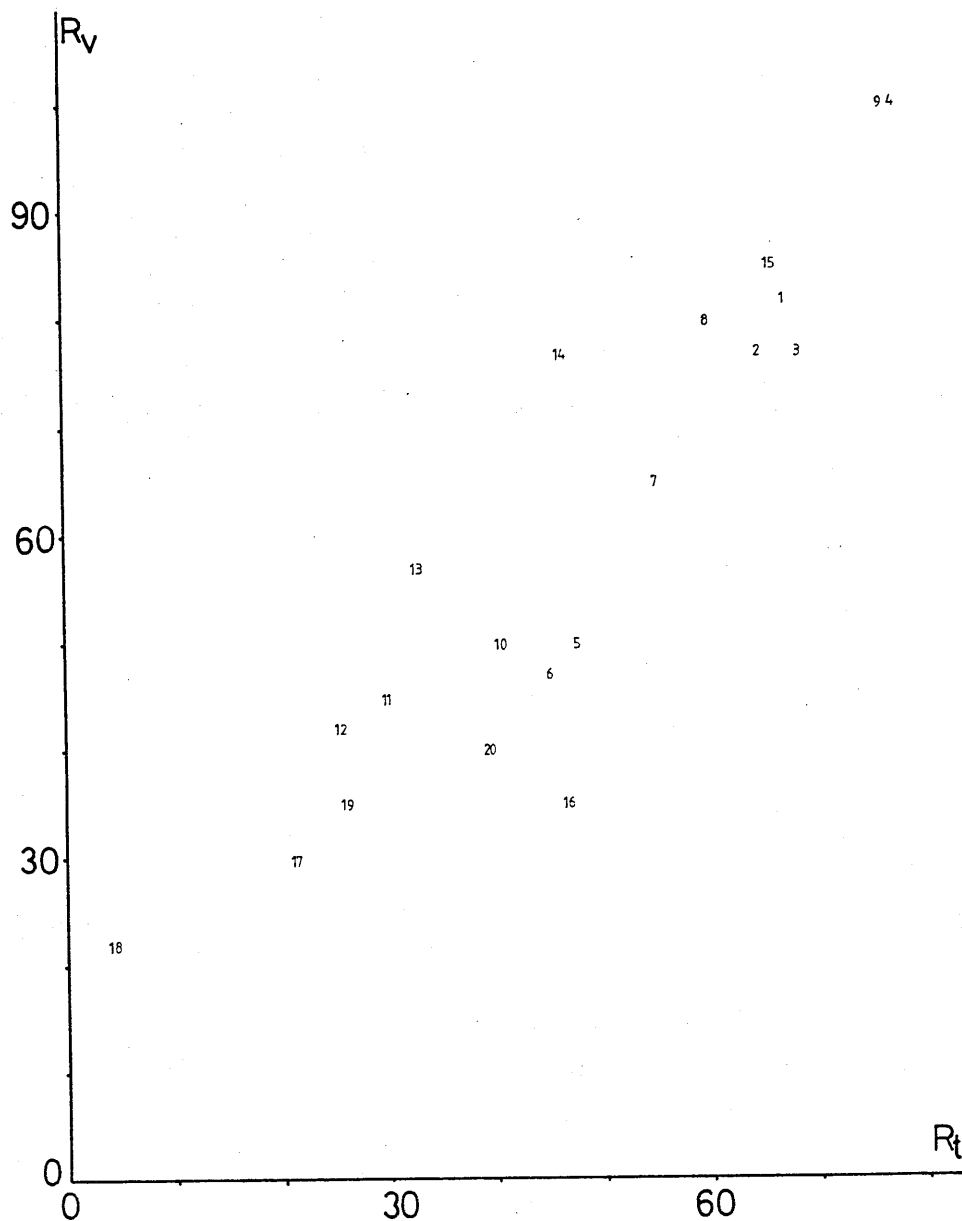
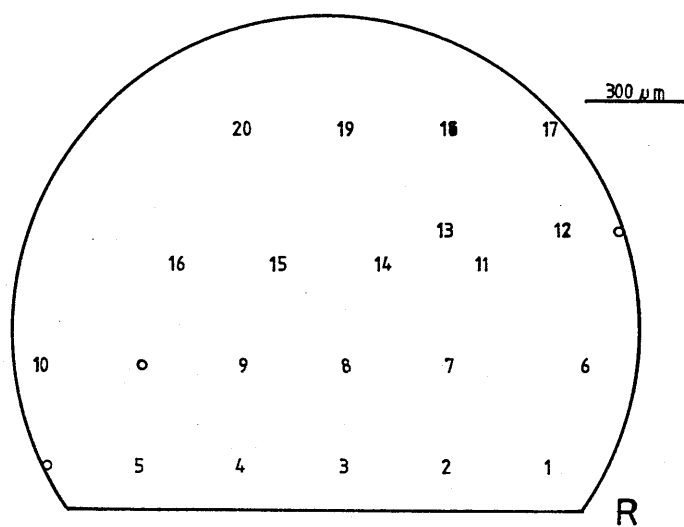
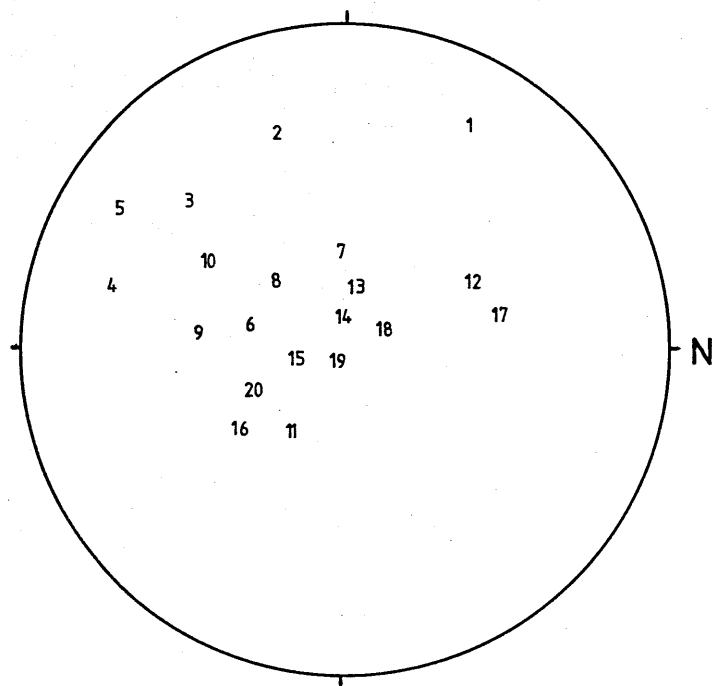


FIGURE 55c



R 58

FIGURE 56a

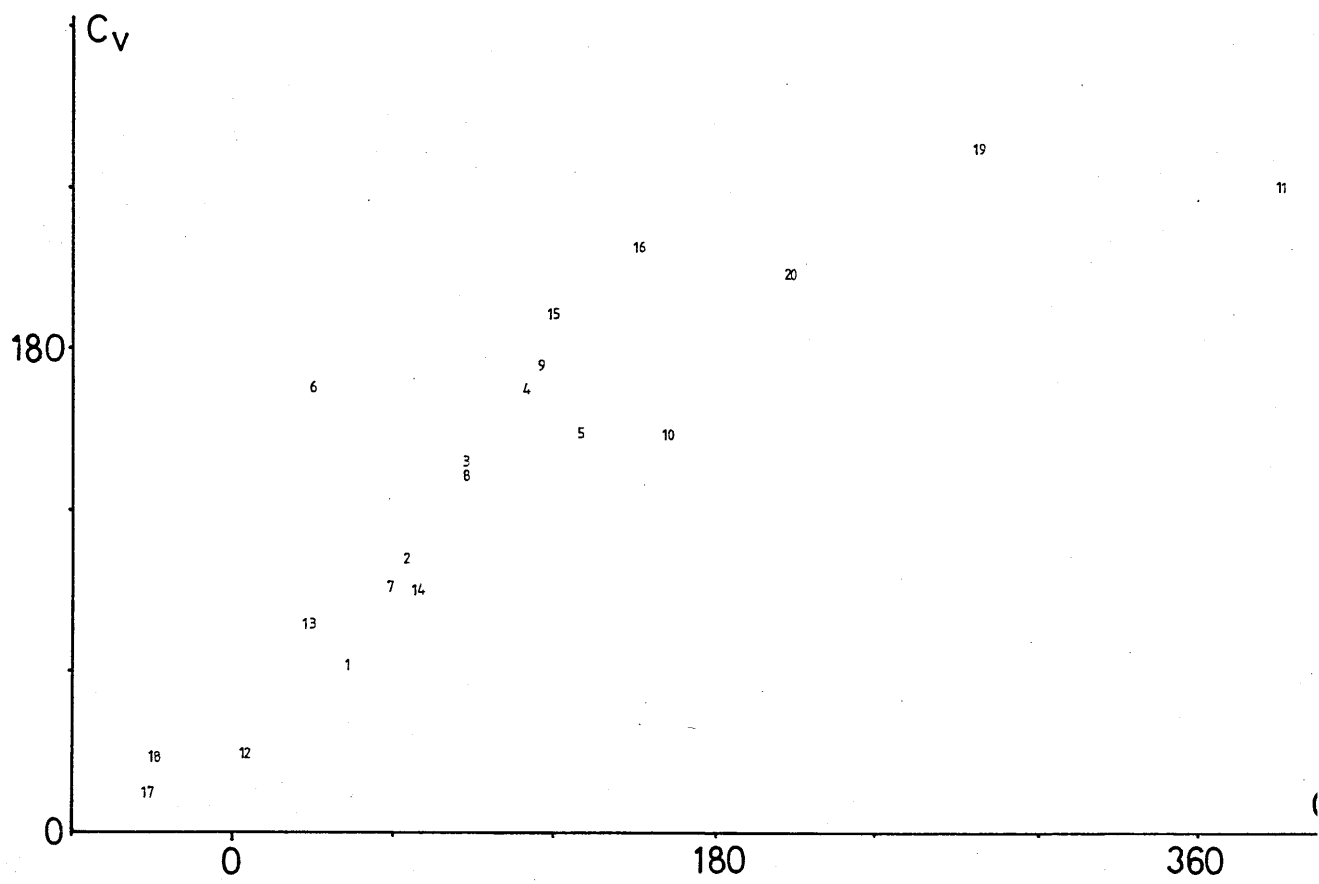


FIGURE 56 b

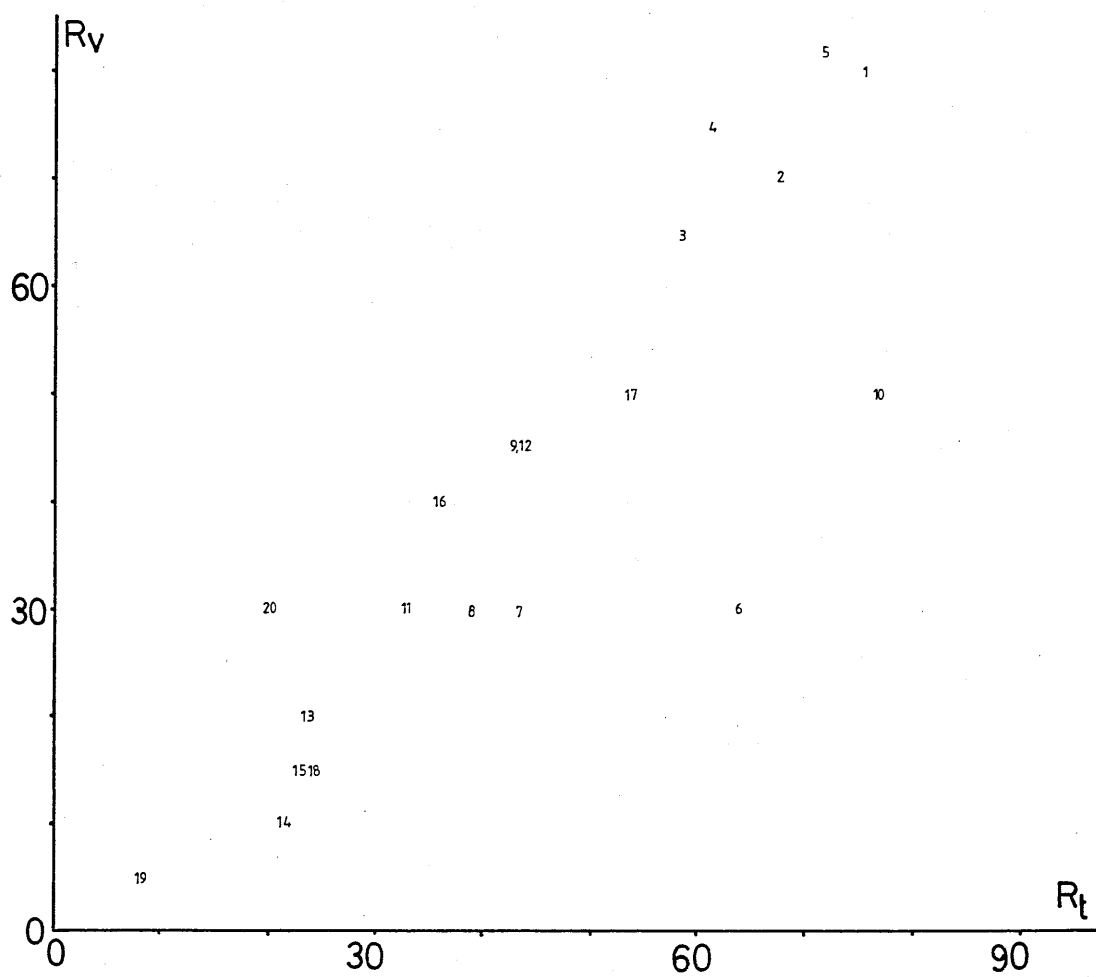
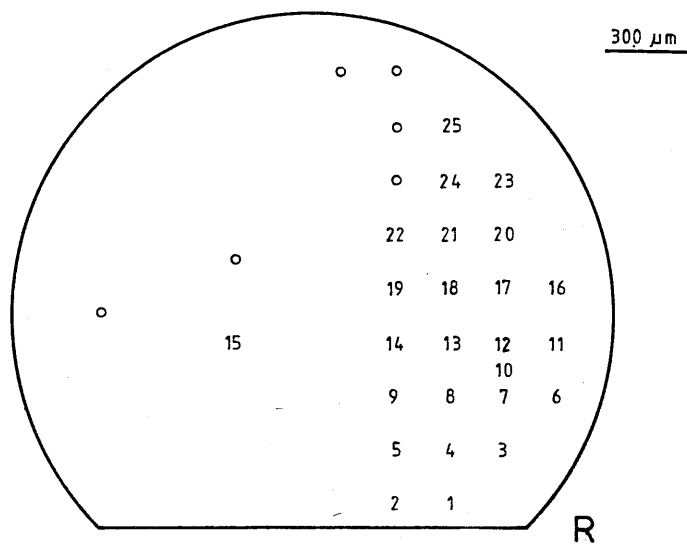
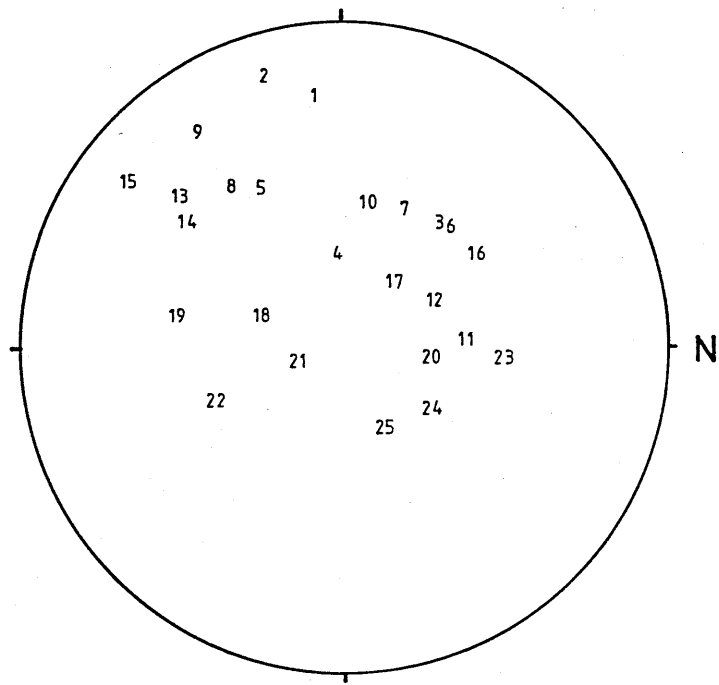


FIGURE 56c





R 70

FIGURE 57a

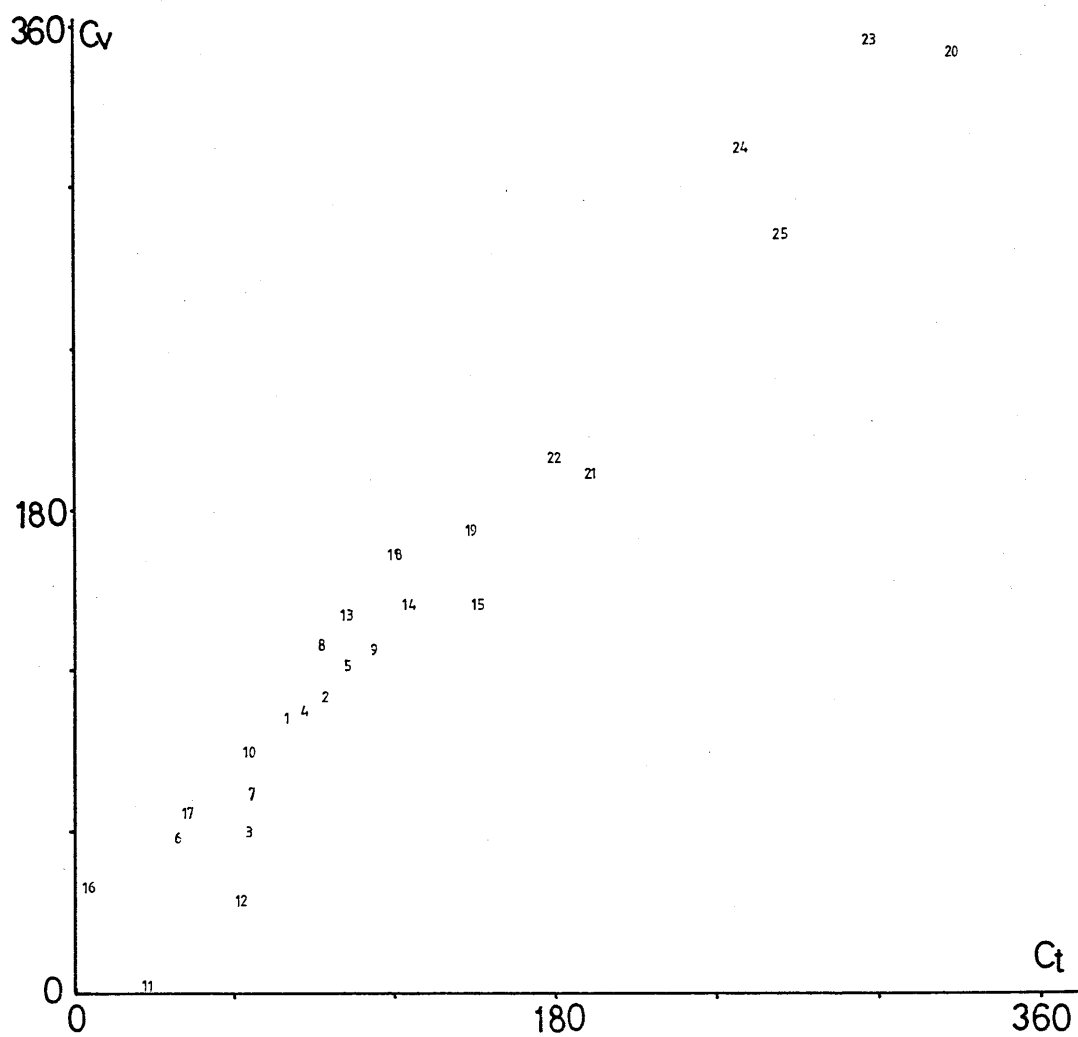


FIGURE 57 b

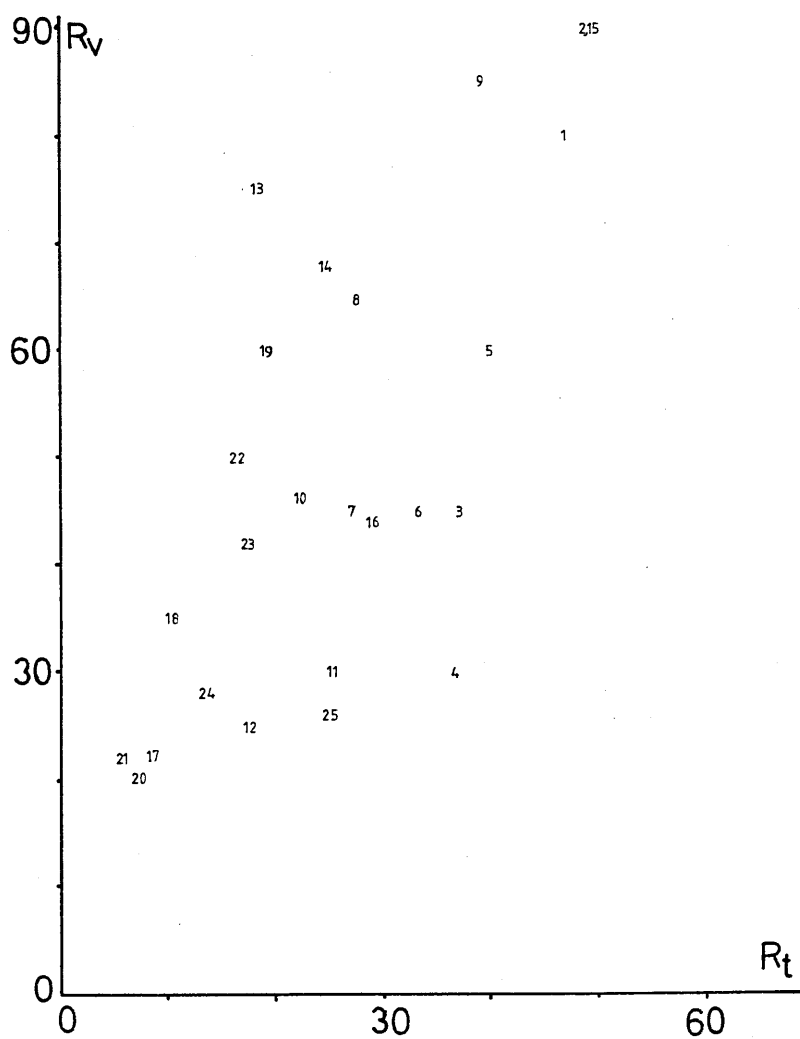
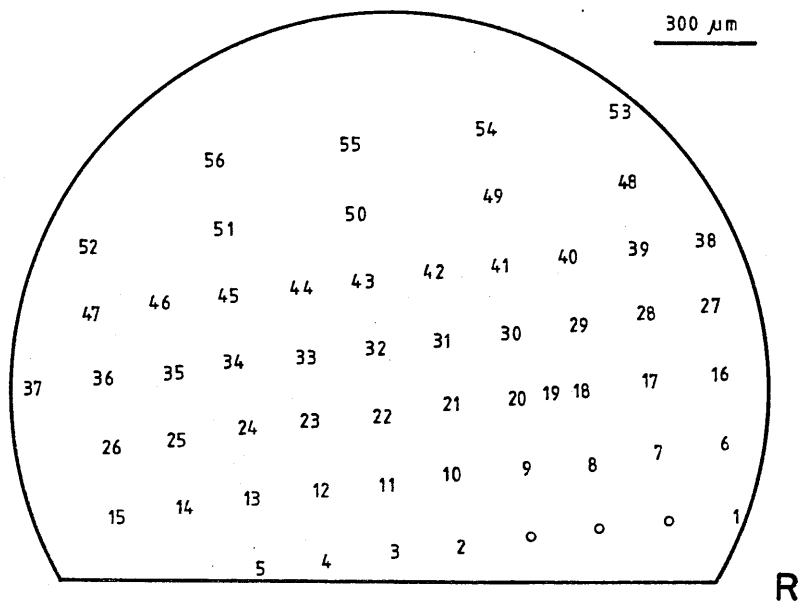
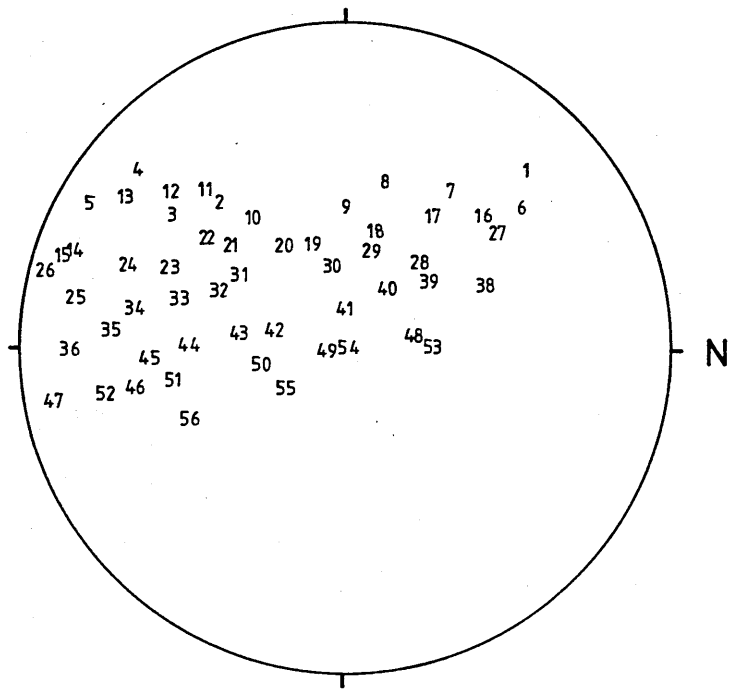


FIGURE 57 c



R 166 A

FIGURE 58a

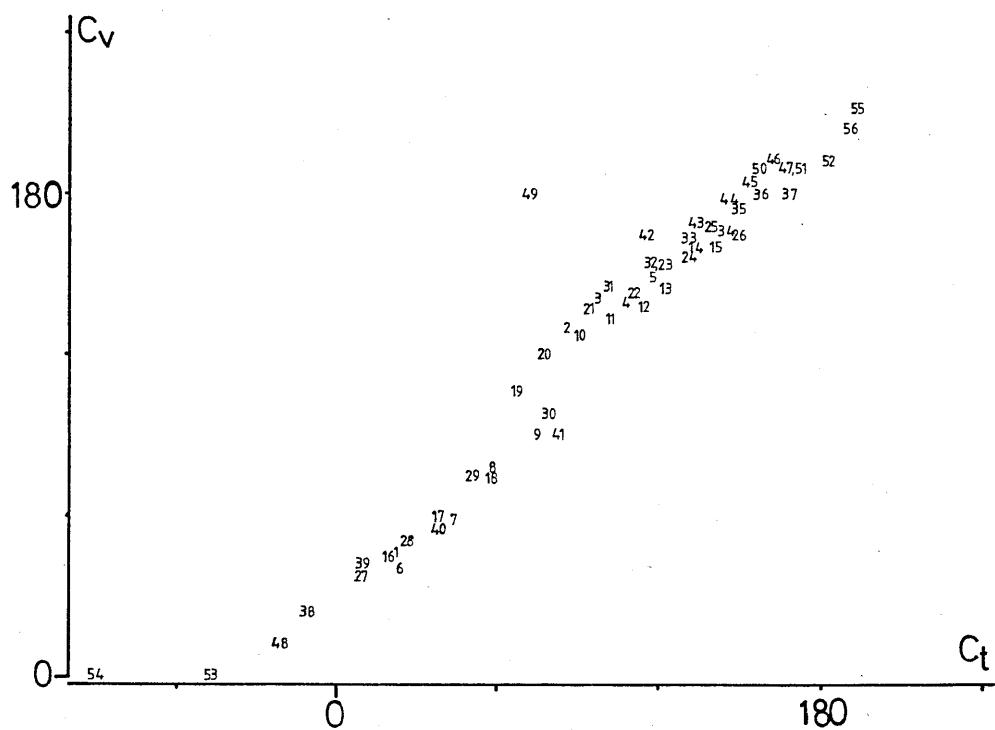


FIGURE 58 b

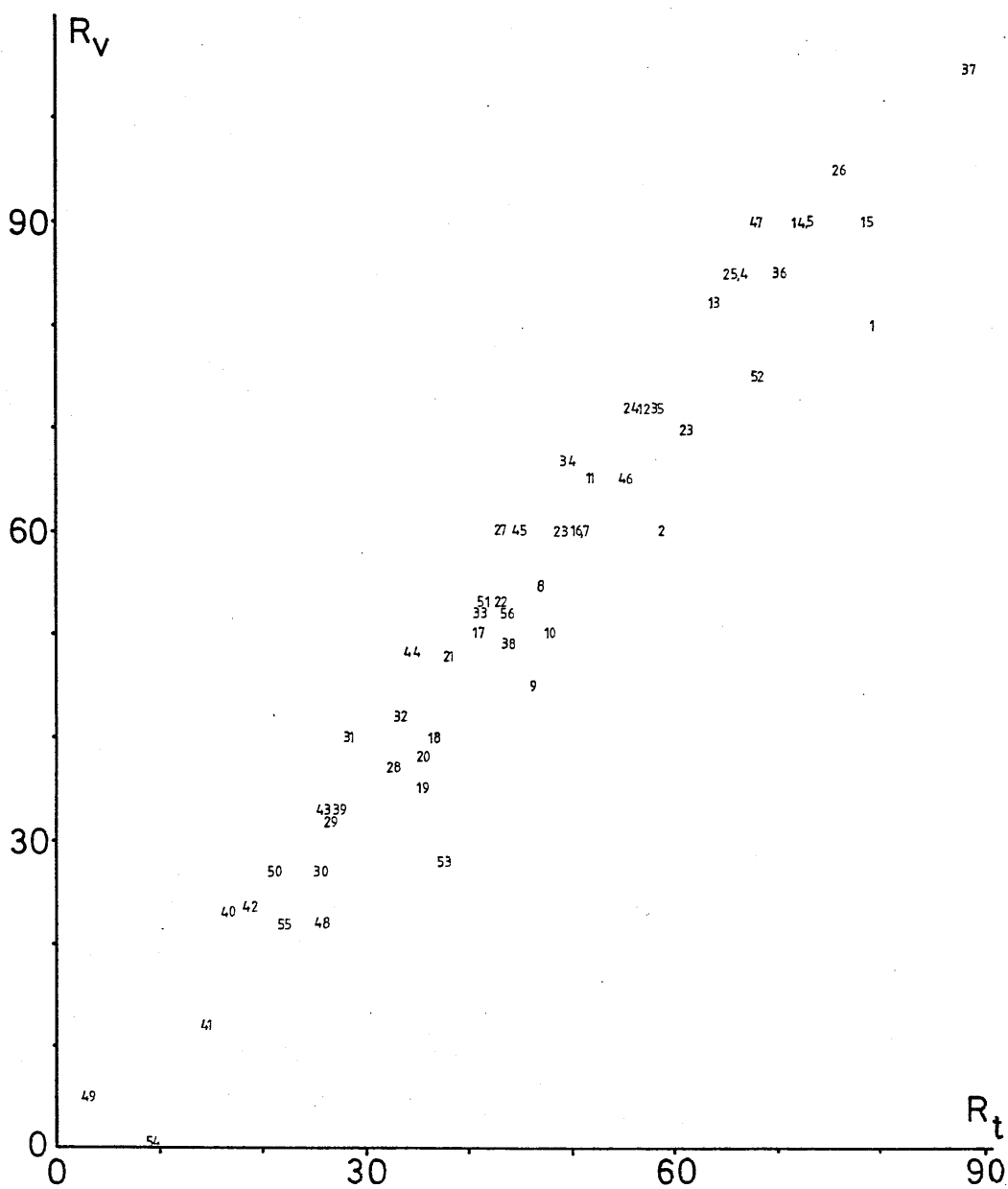
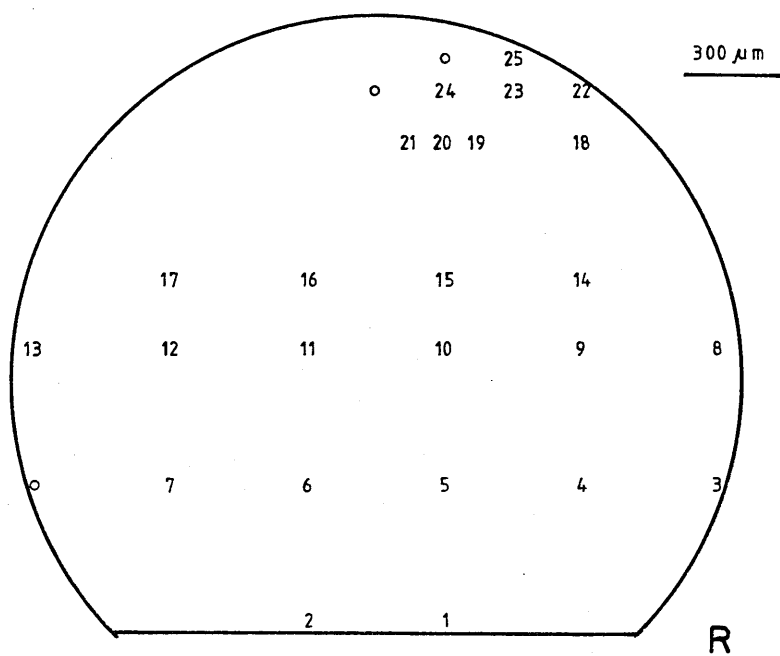
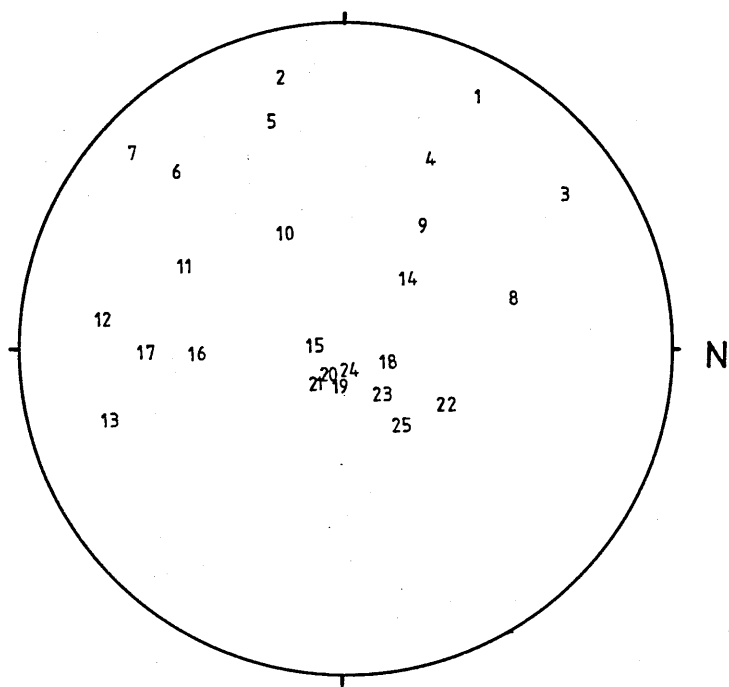


FIGURE 58 c



R 166 B

FIGURE 59a

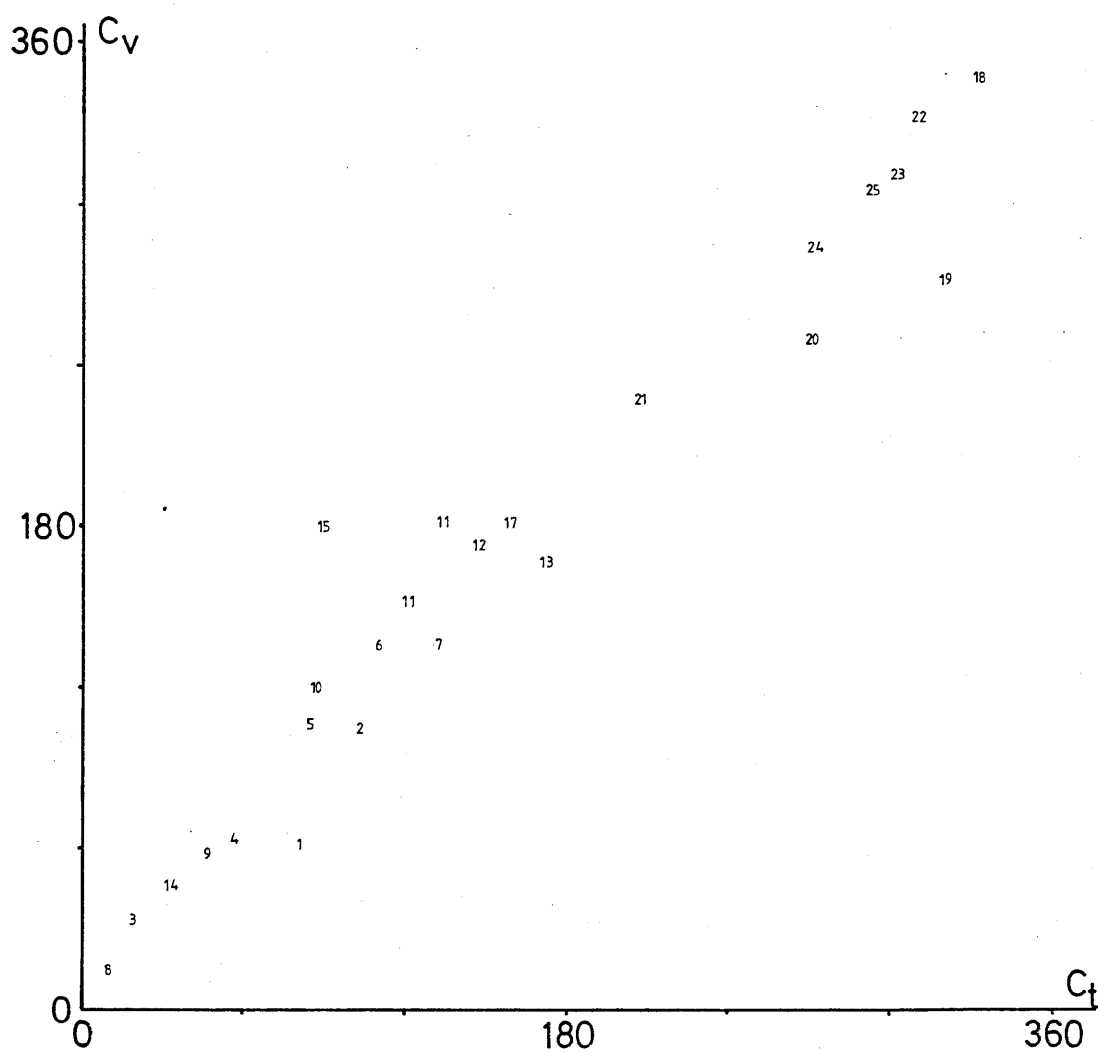


FIGURE 59 b



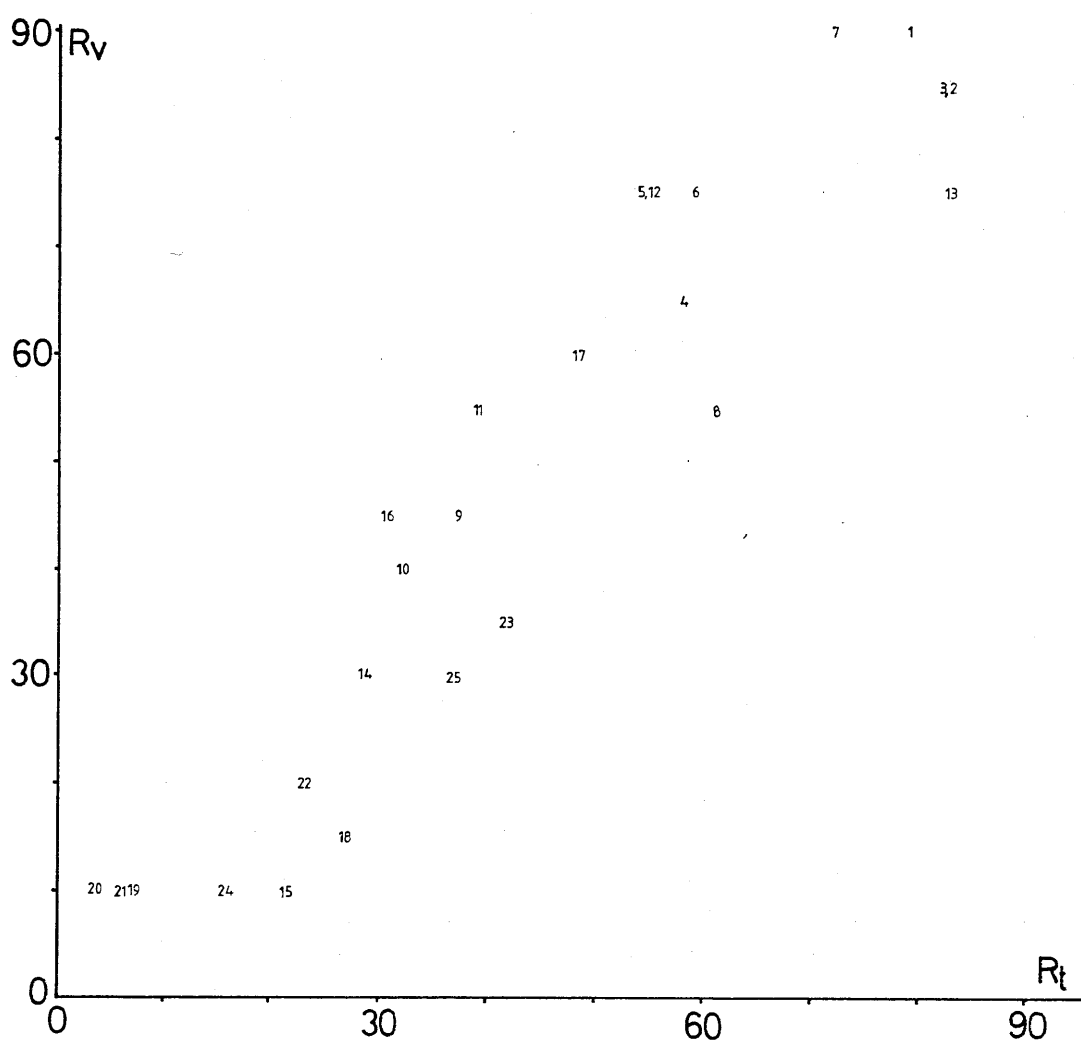
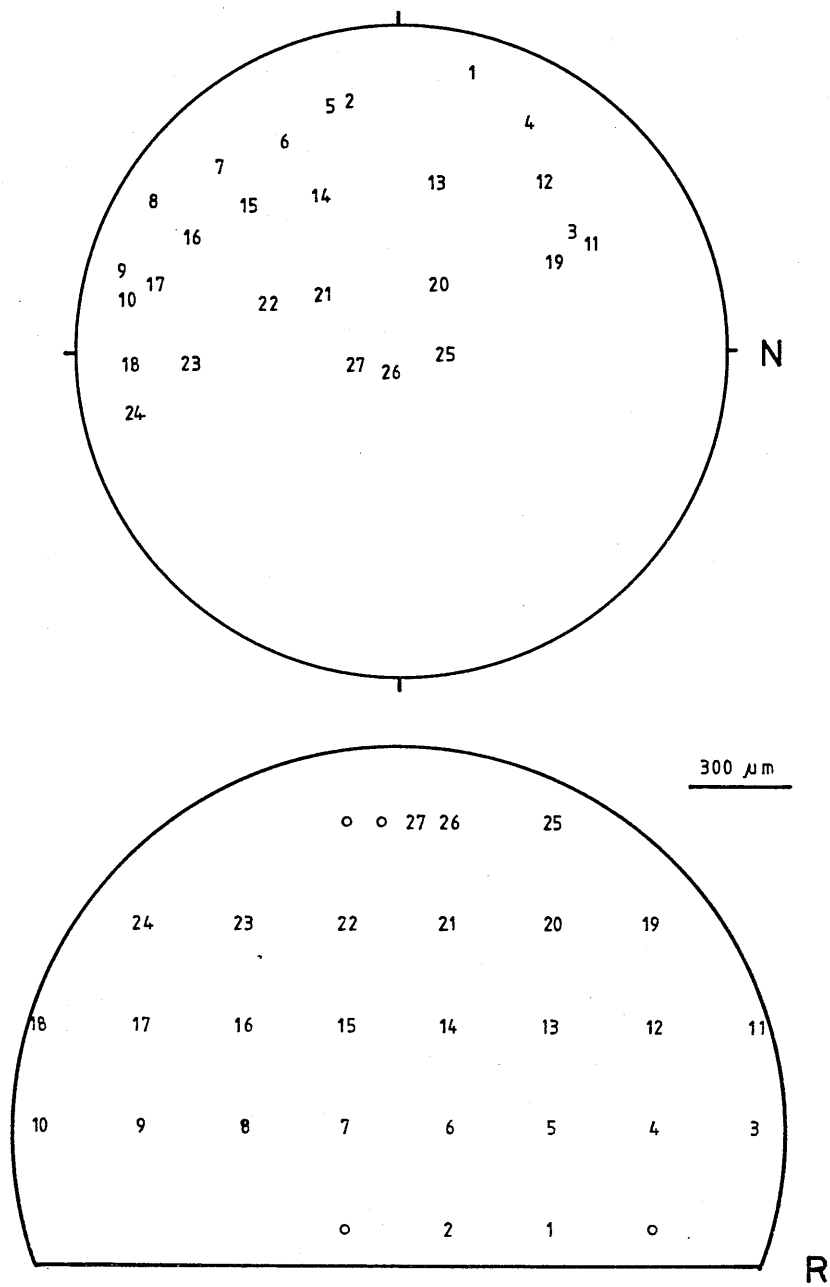


FIGURE 59c



R 167

FIGURE 60a

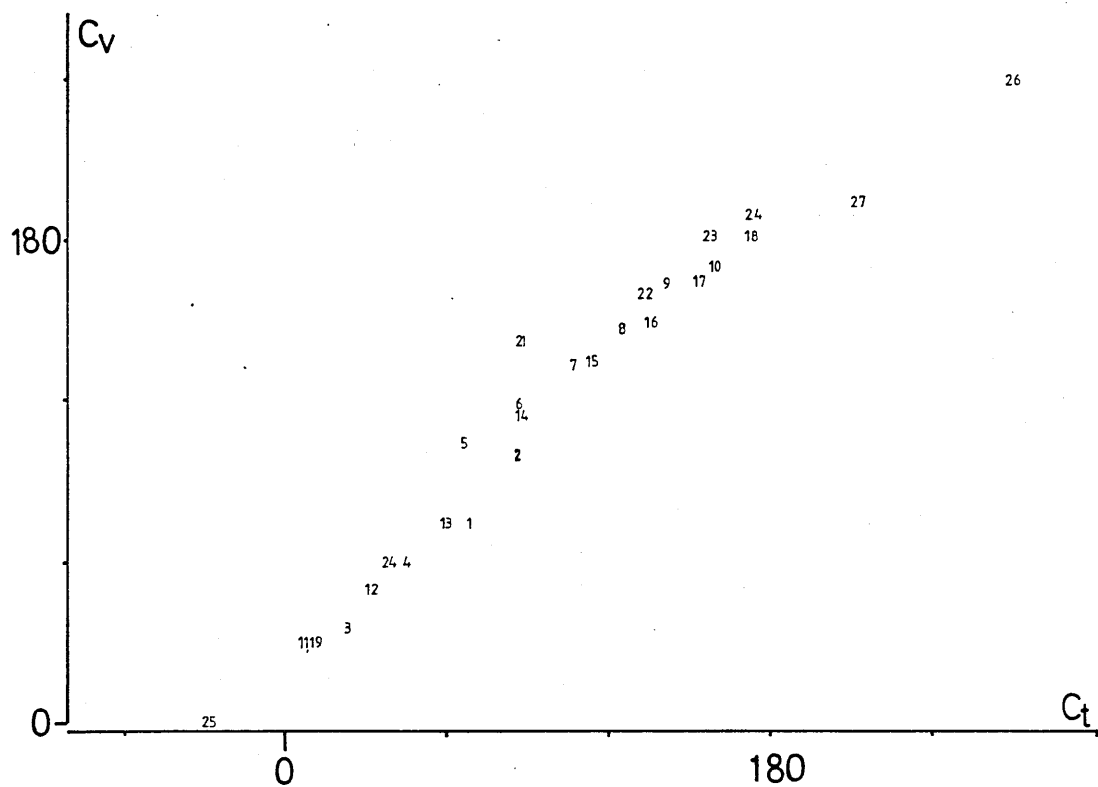


FIGURE 60b

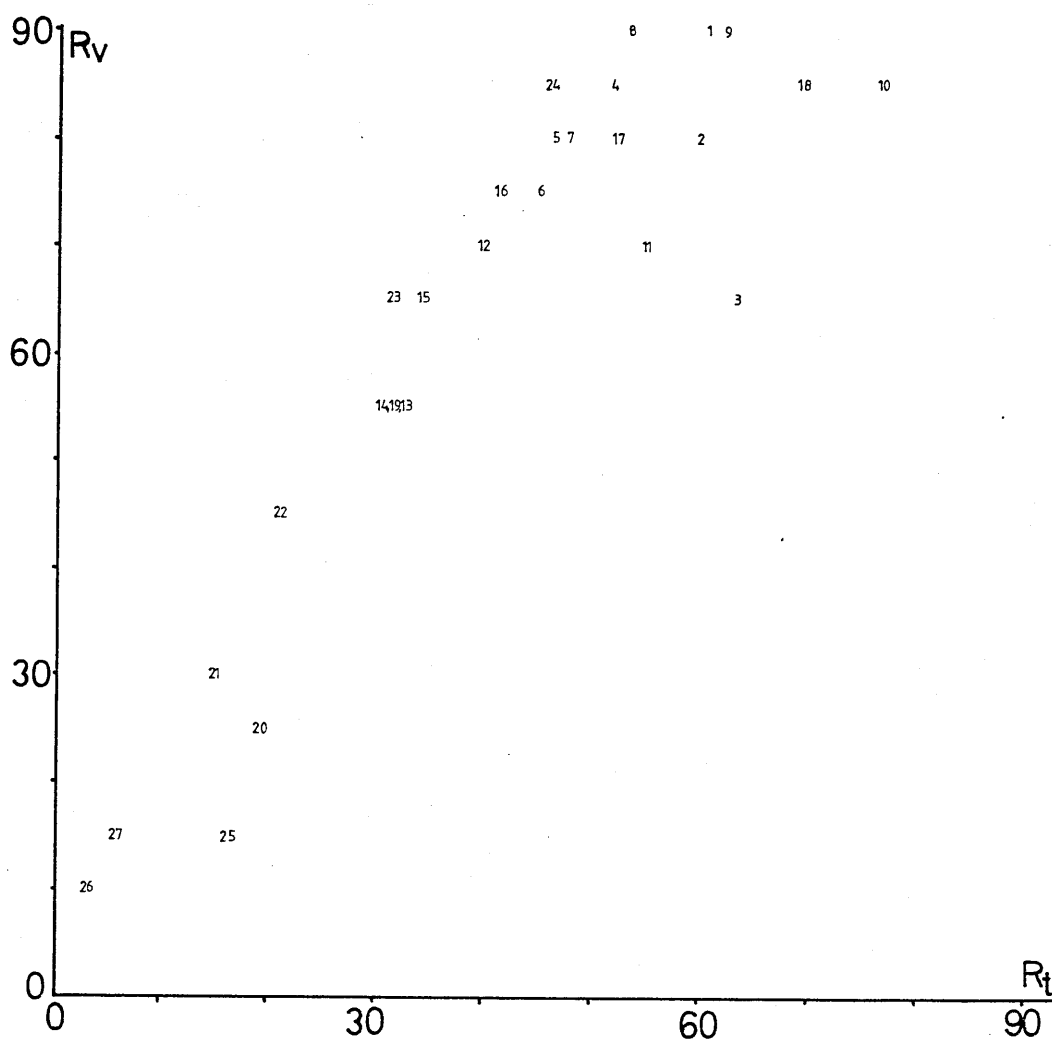


FIGURE 60c

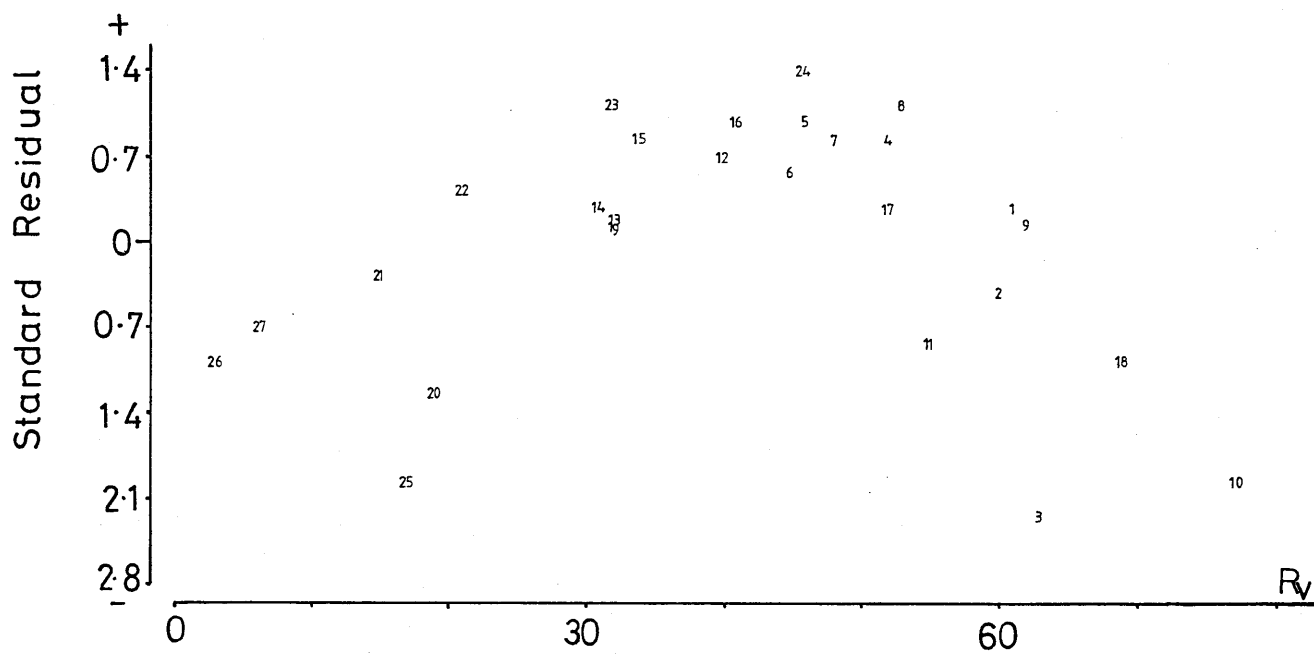


FIGURE 60 d

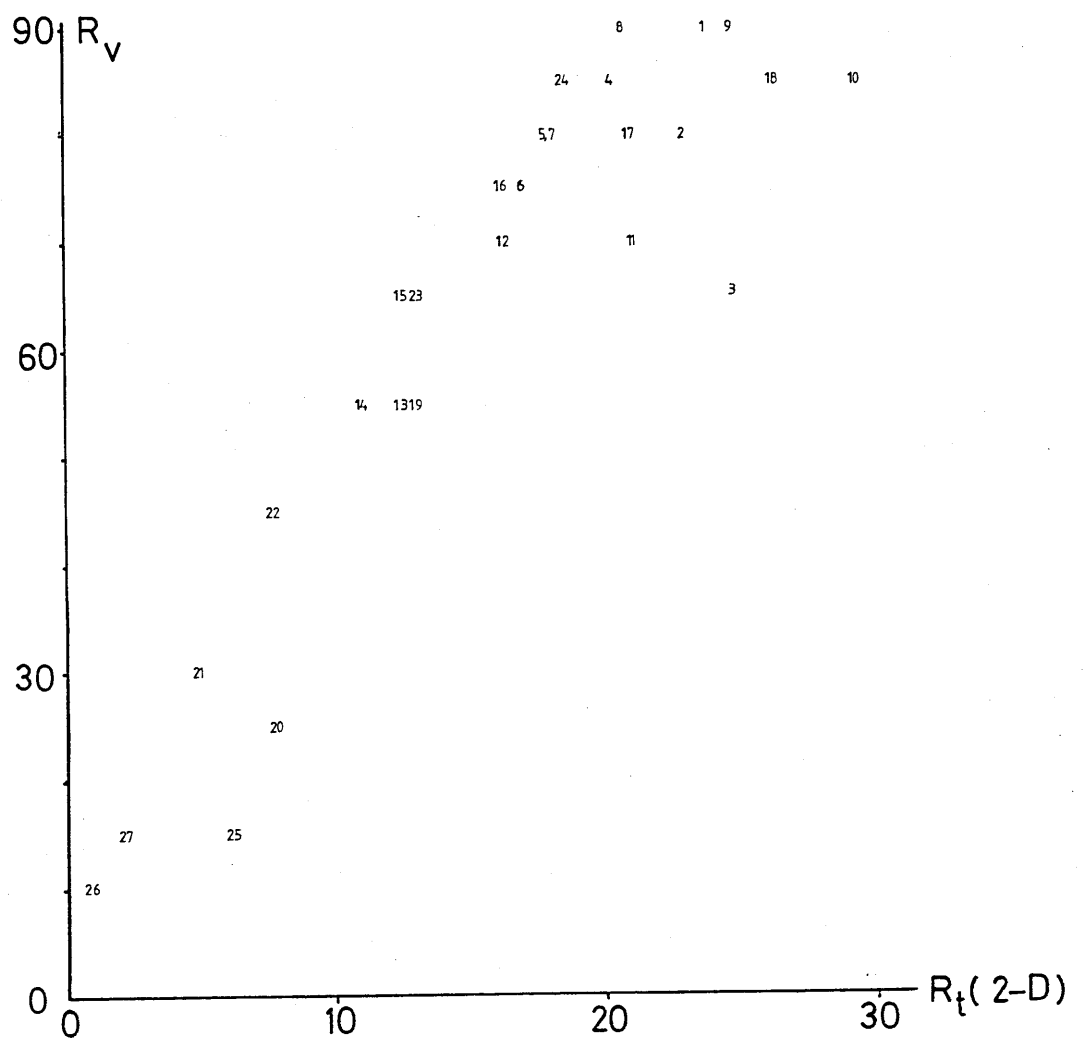
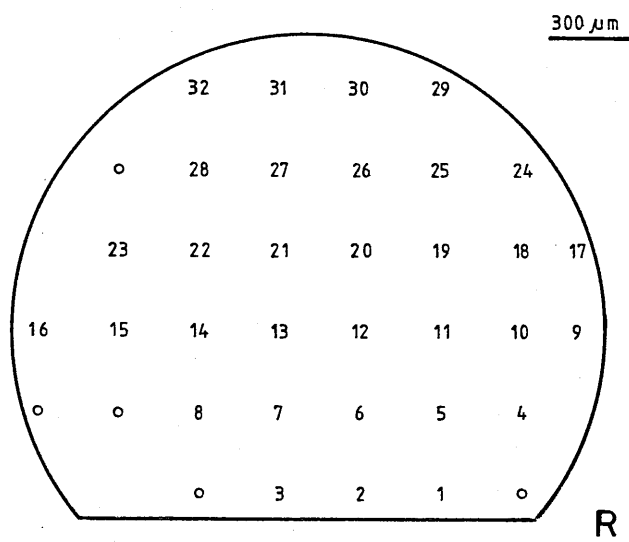
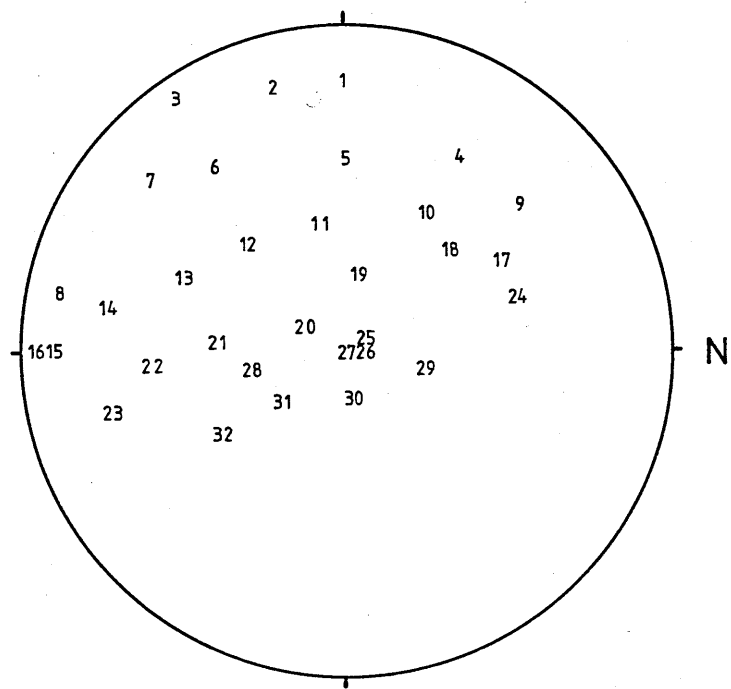


FIGURE 60 e



R168A

FIGURE 61a

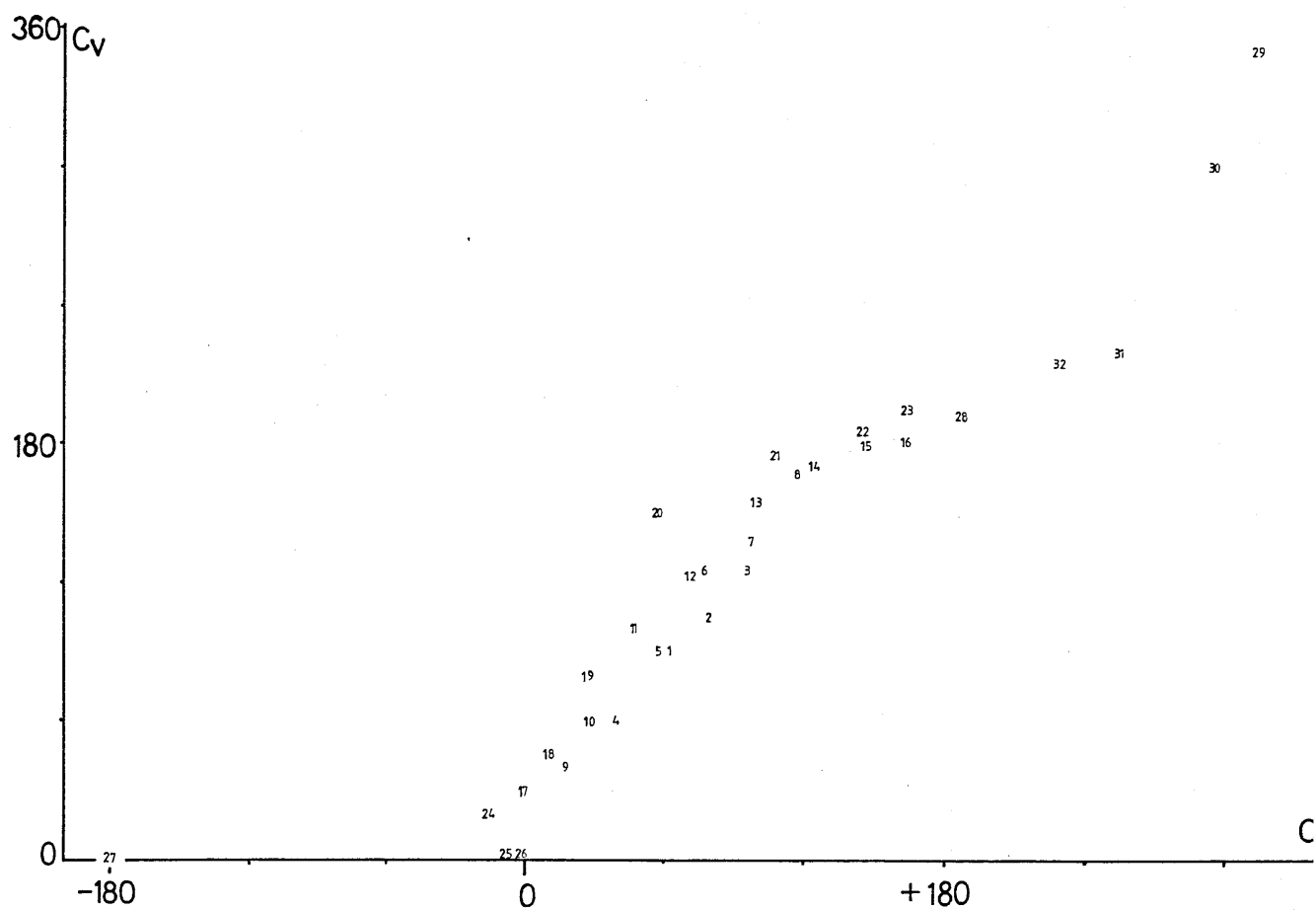


FIGURE 61b



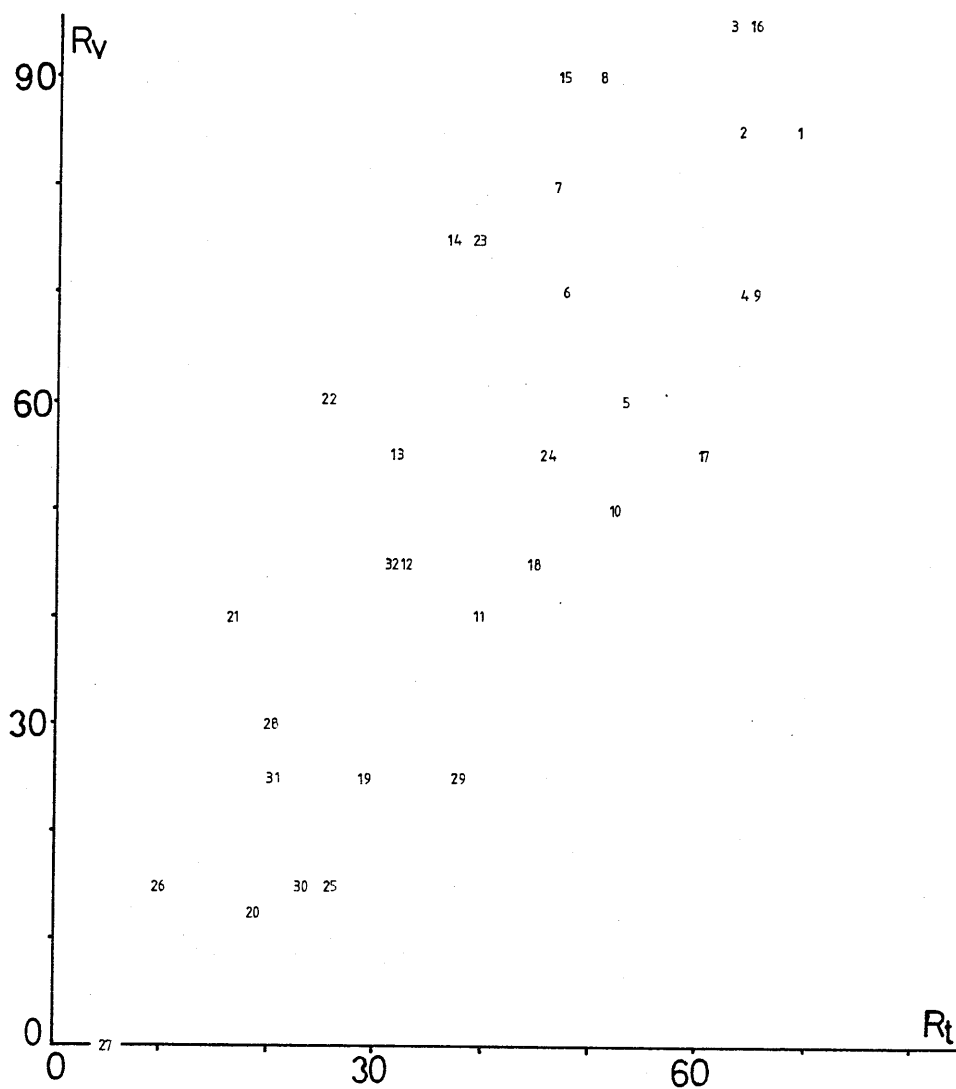


FIGURE 61c

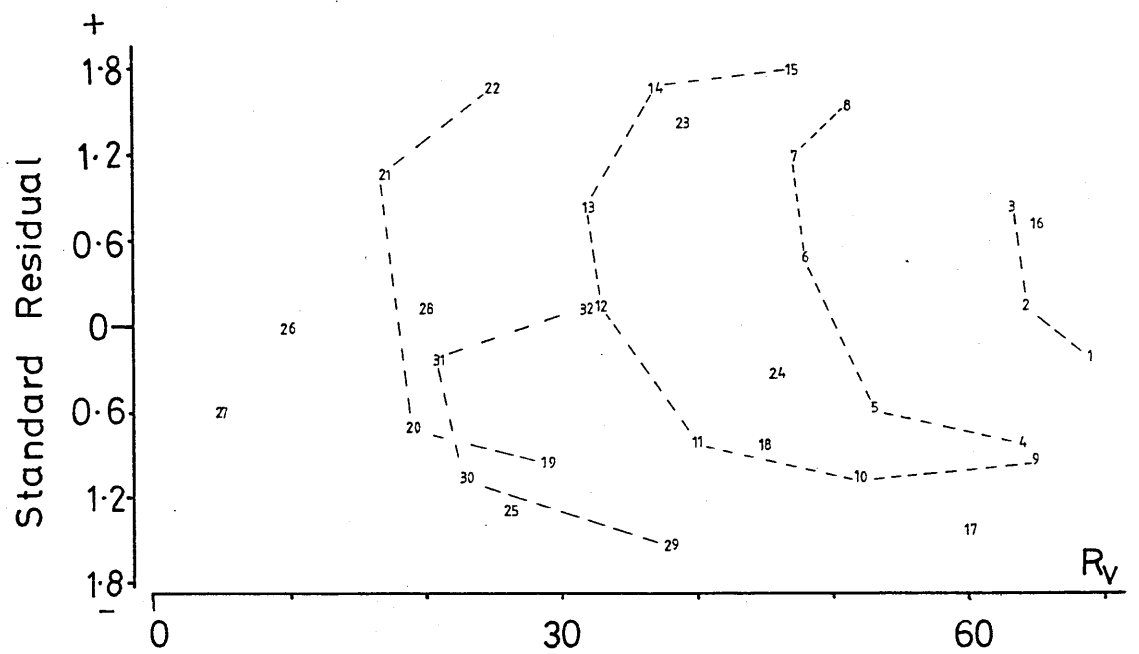


FIGURE 61 d

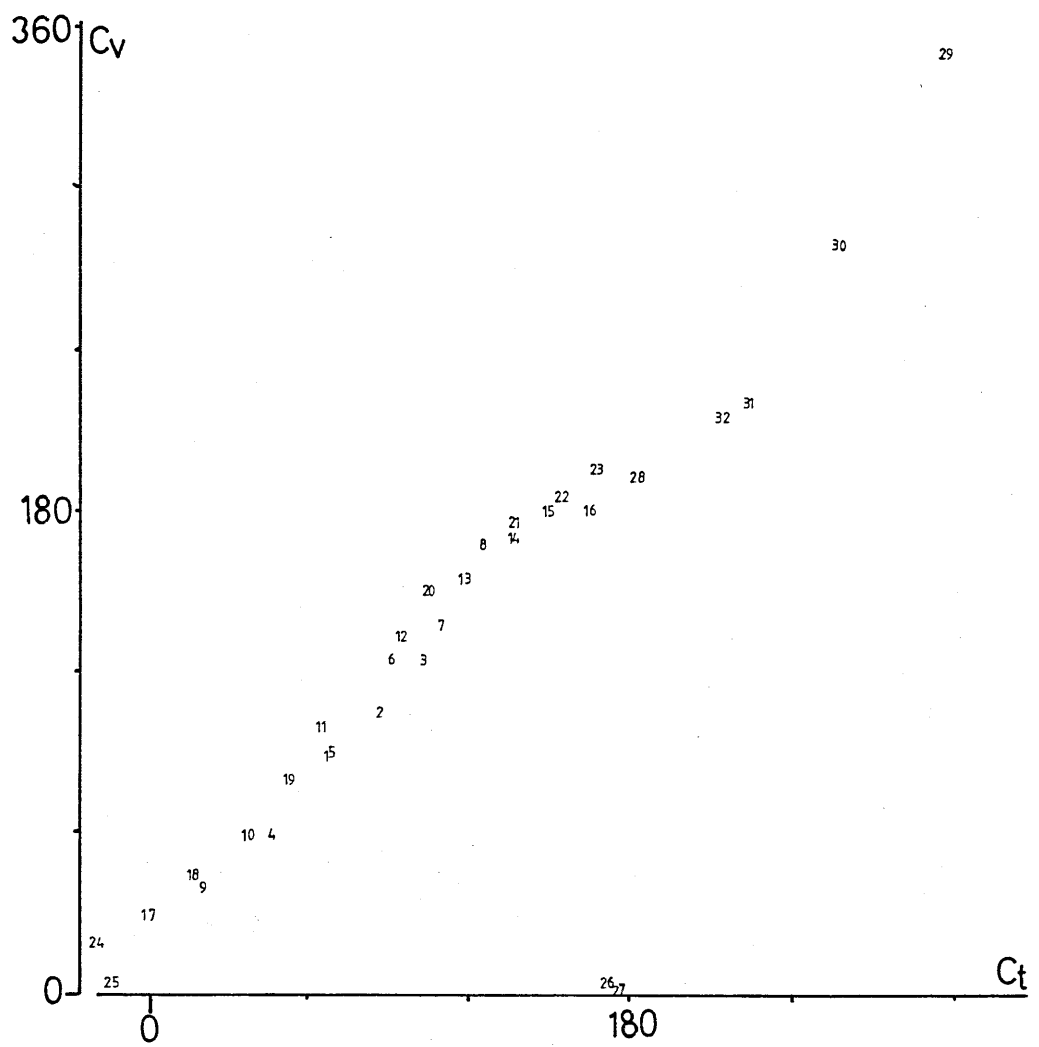


FIGURE 61 e

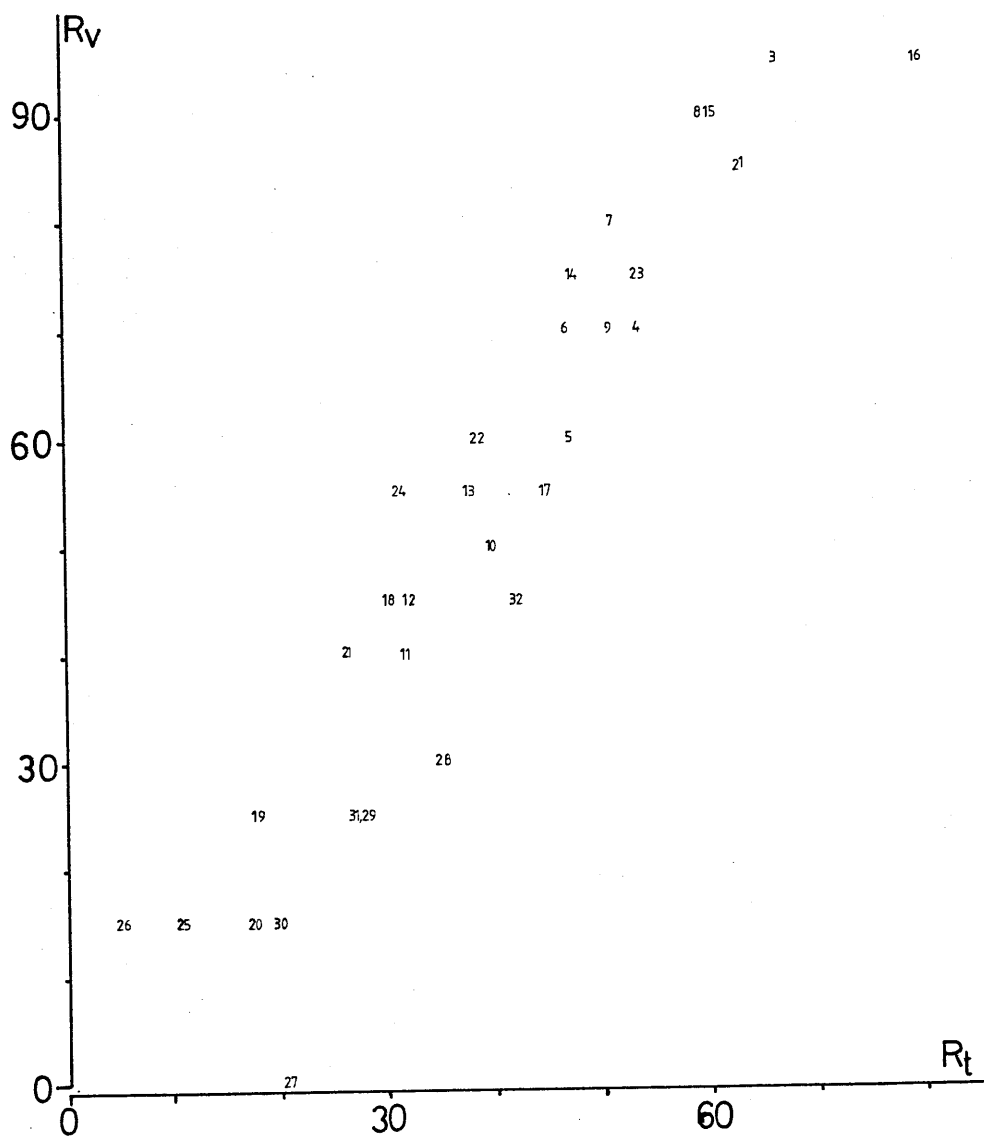
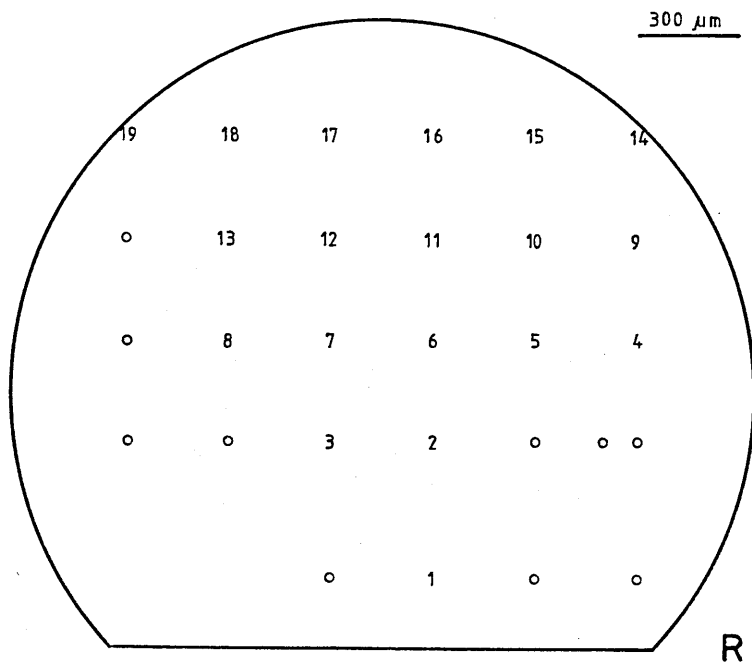
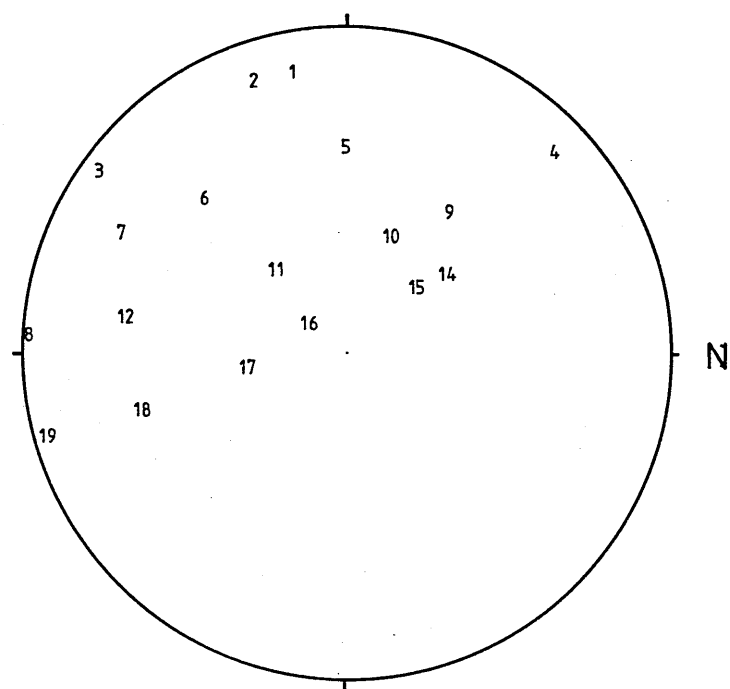


FIGURE 61f



R168B

FIGURE 62a

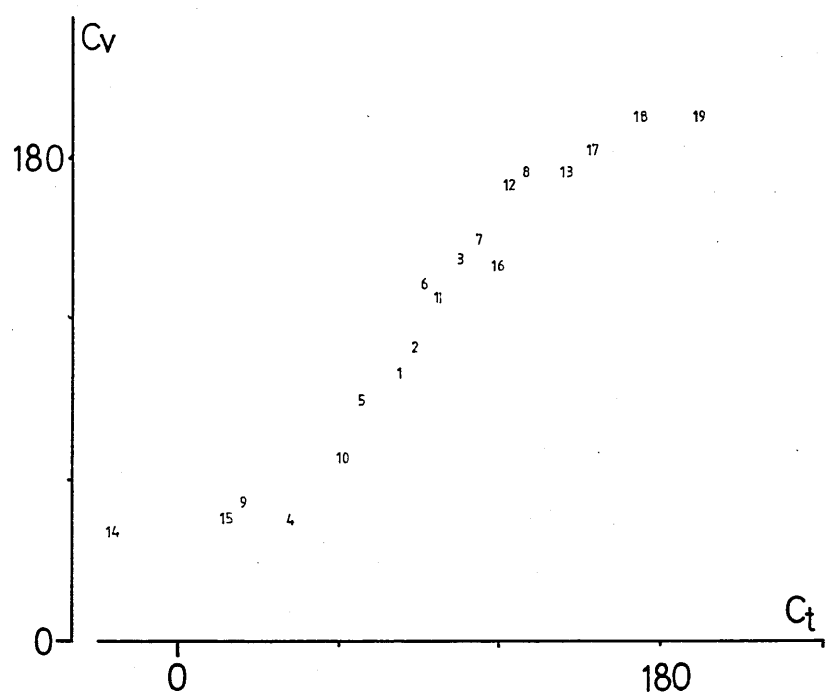


FIGURE 62 b

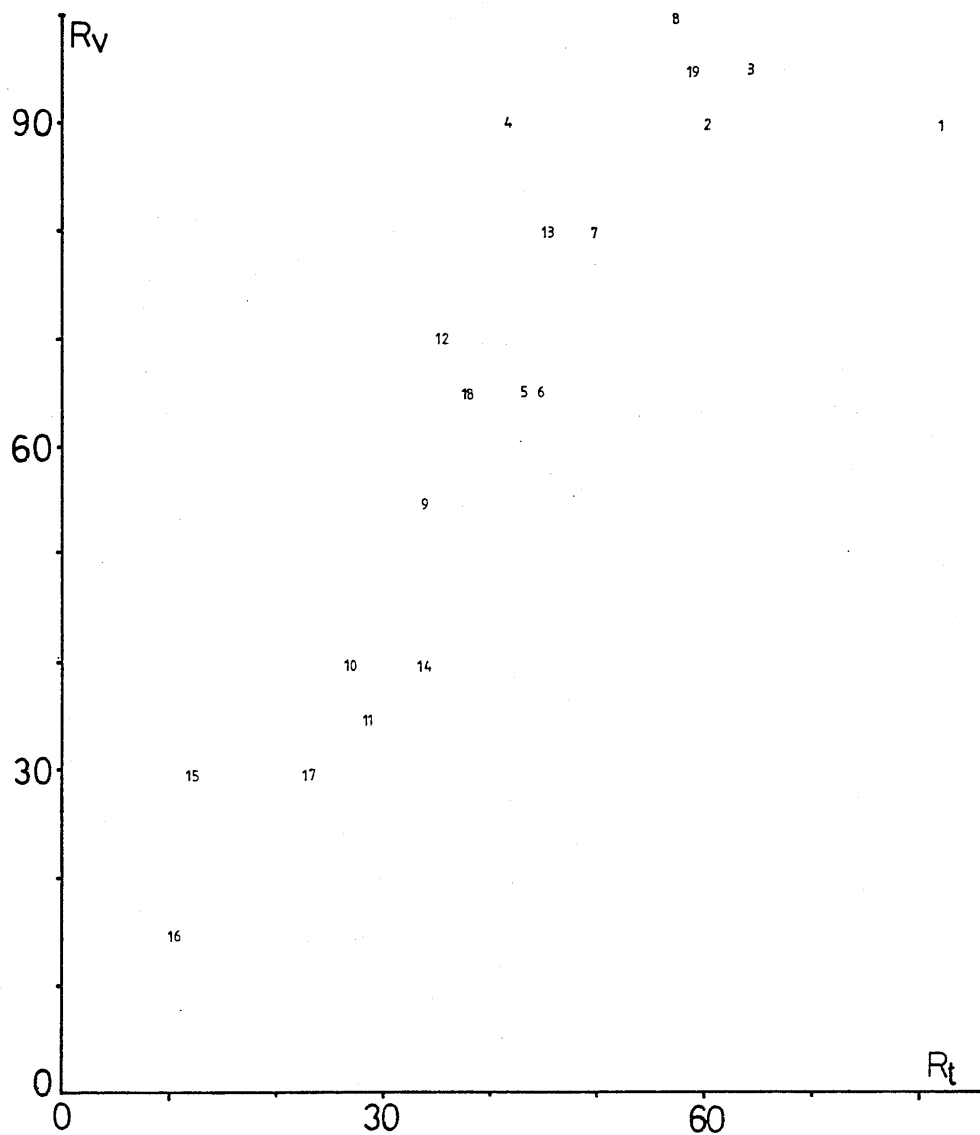
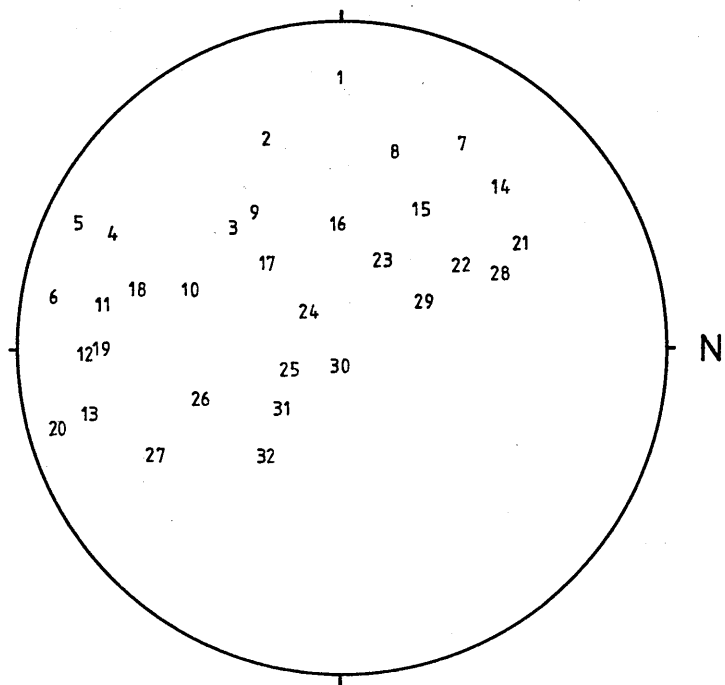
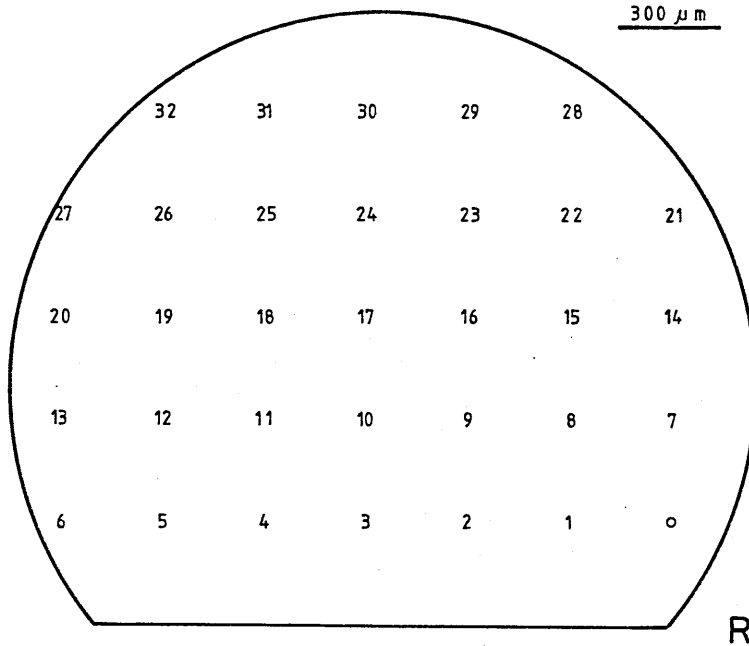


FIGURE 62c



300  $\mu$ m



R168 C

FIGURE 63a



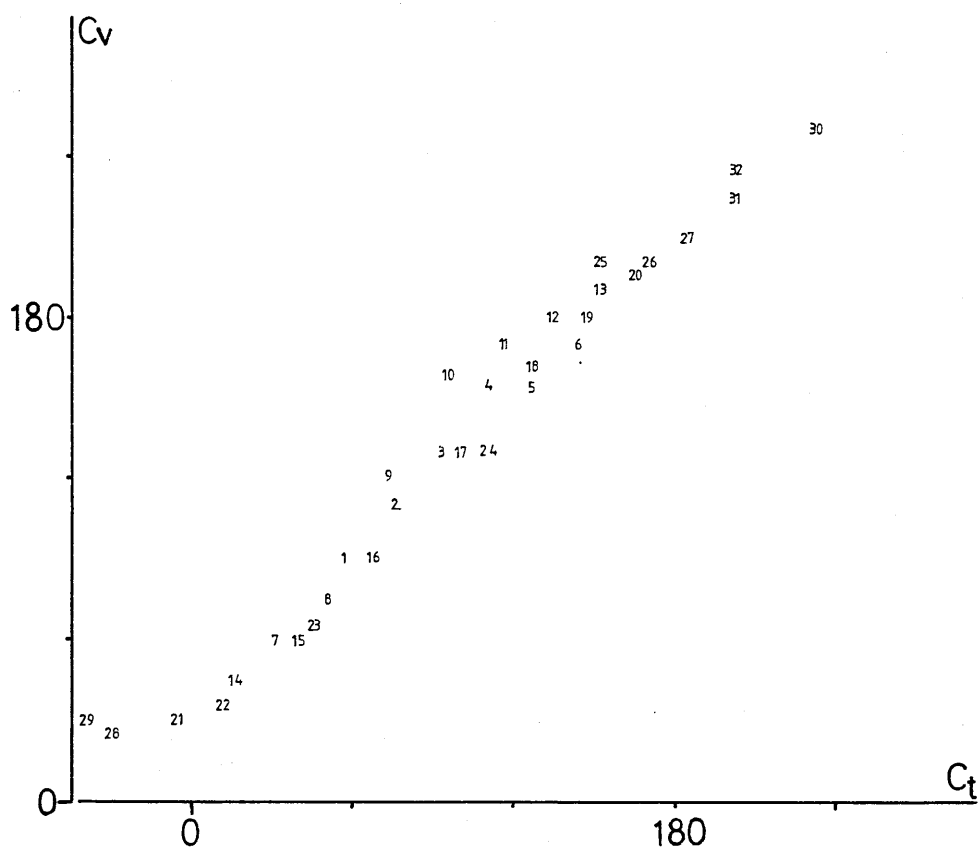


FIGURE 63 b

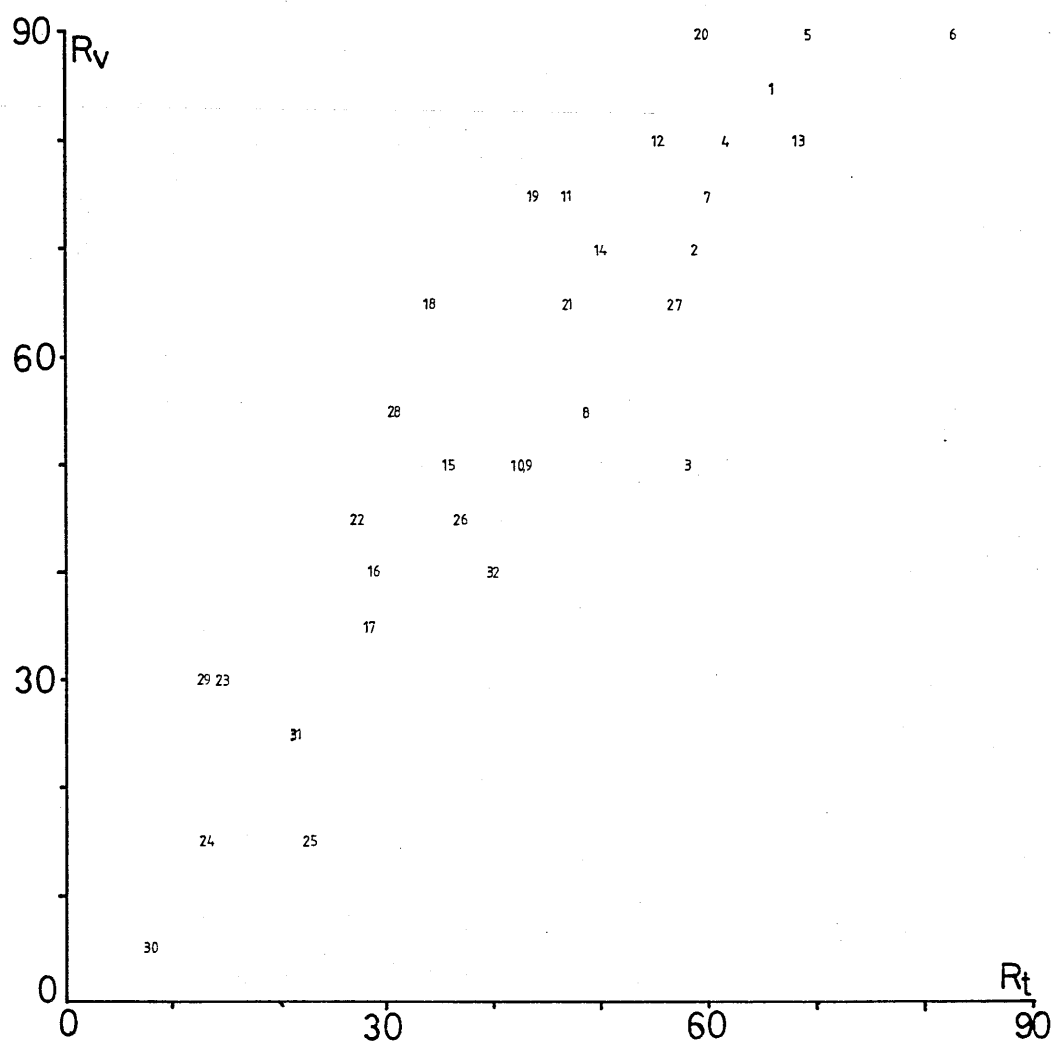


FIGURE 63c

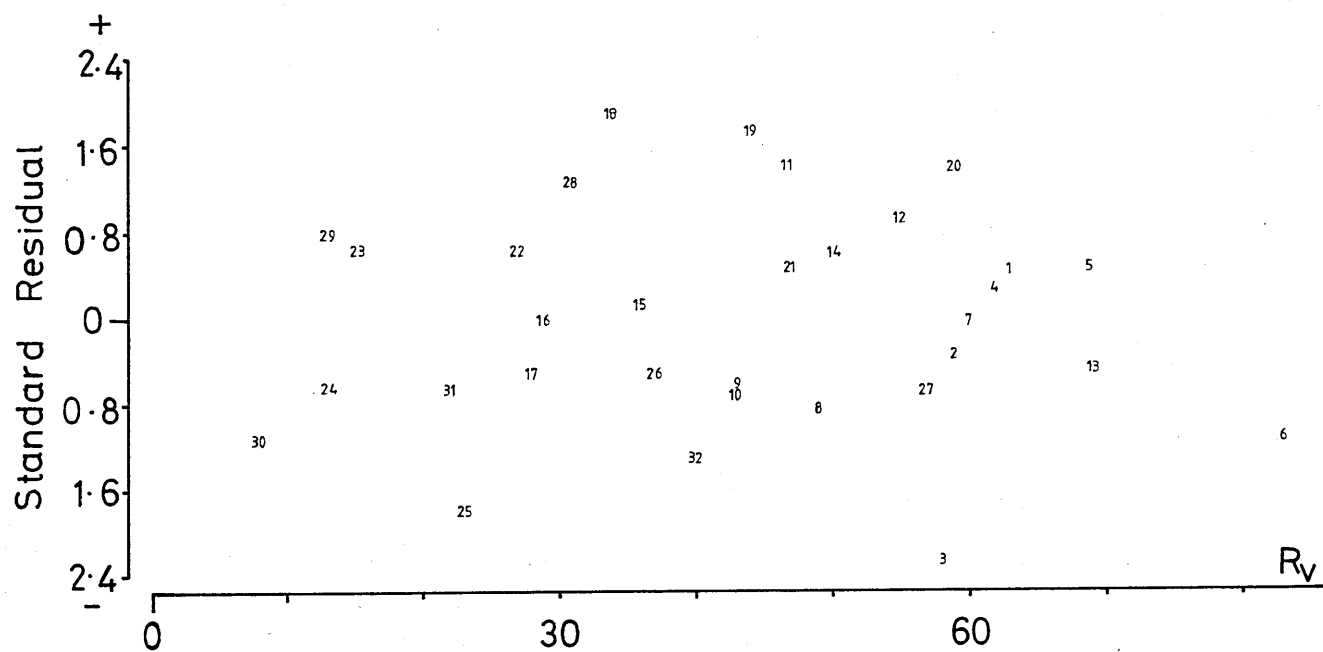
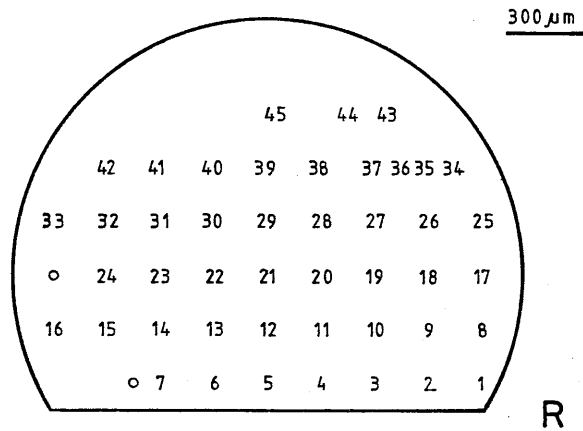
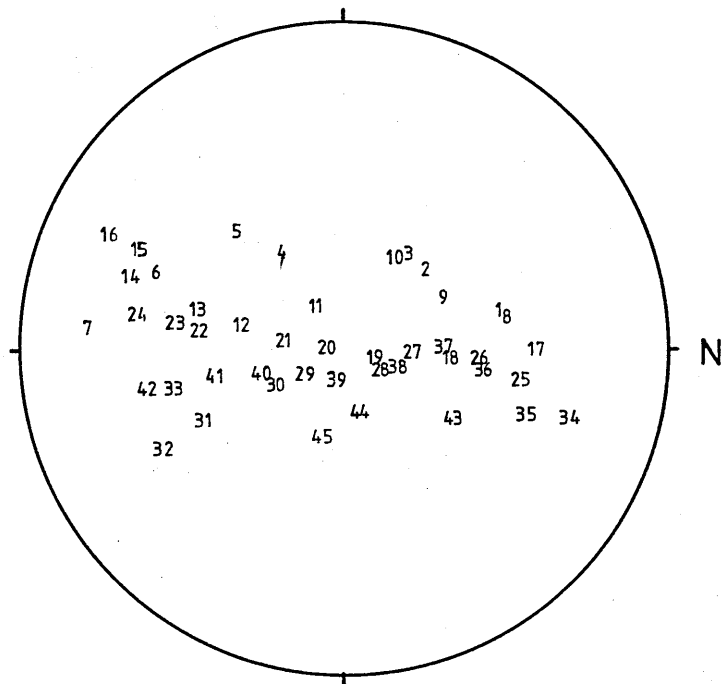


FIGURE 63d



A17D 56

FIGURE 64a

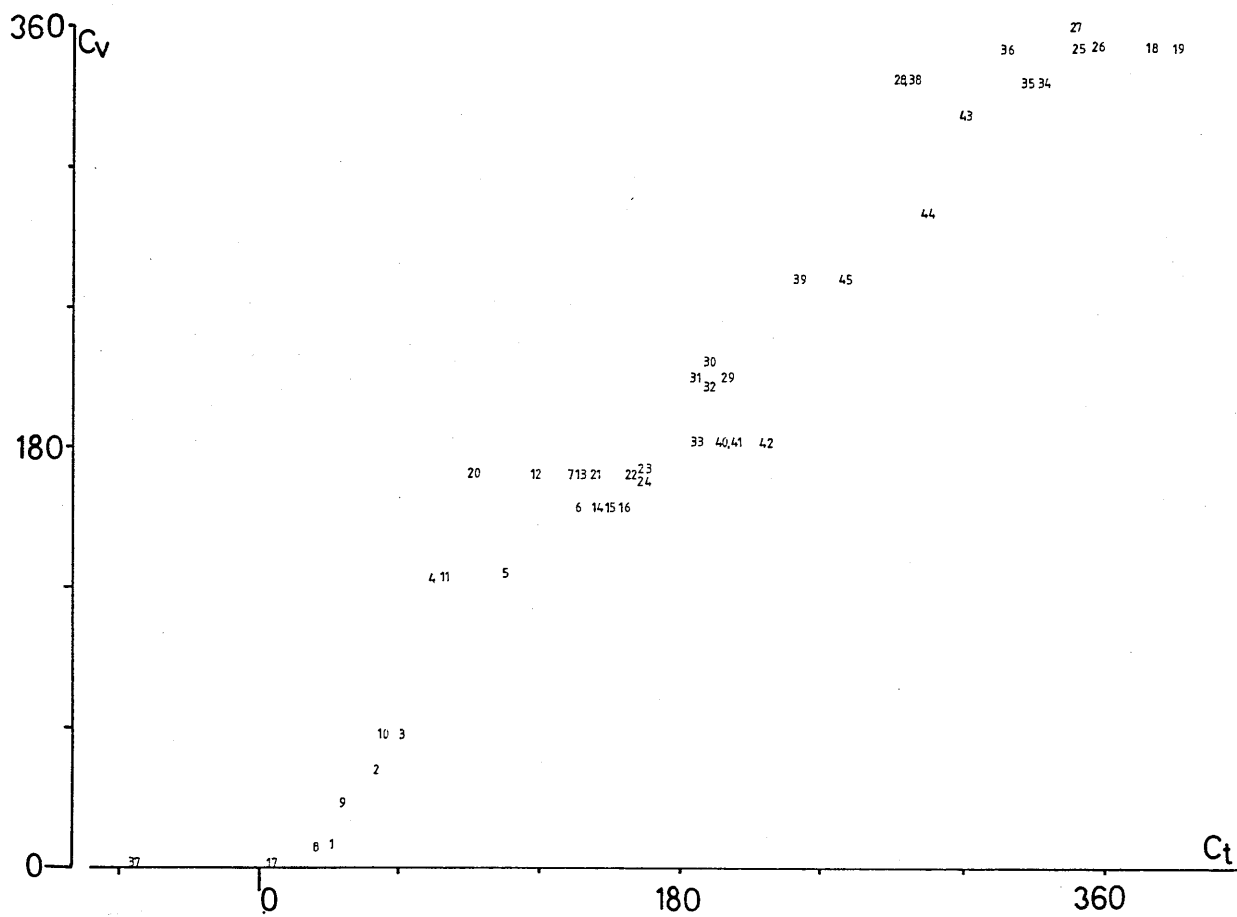


FIGURE 64 b

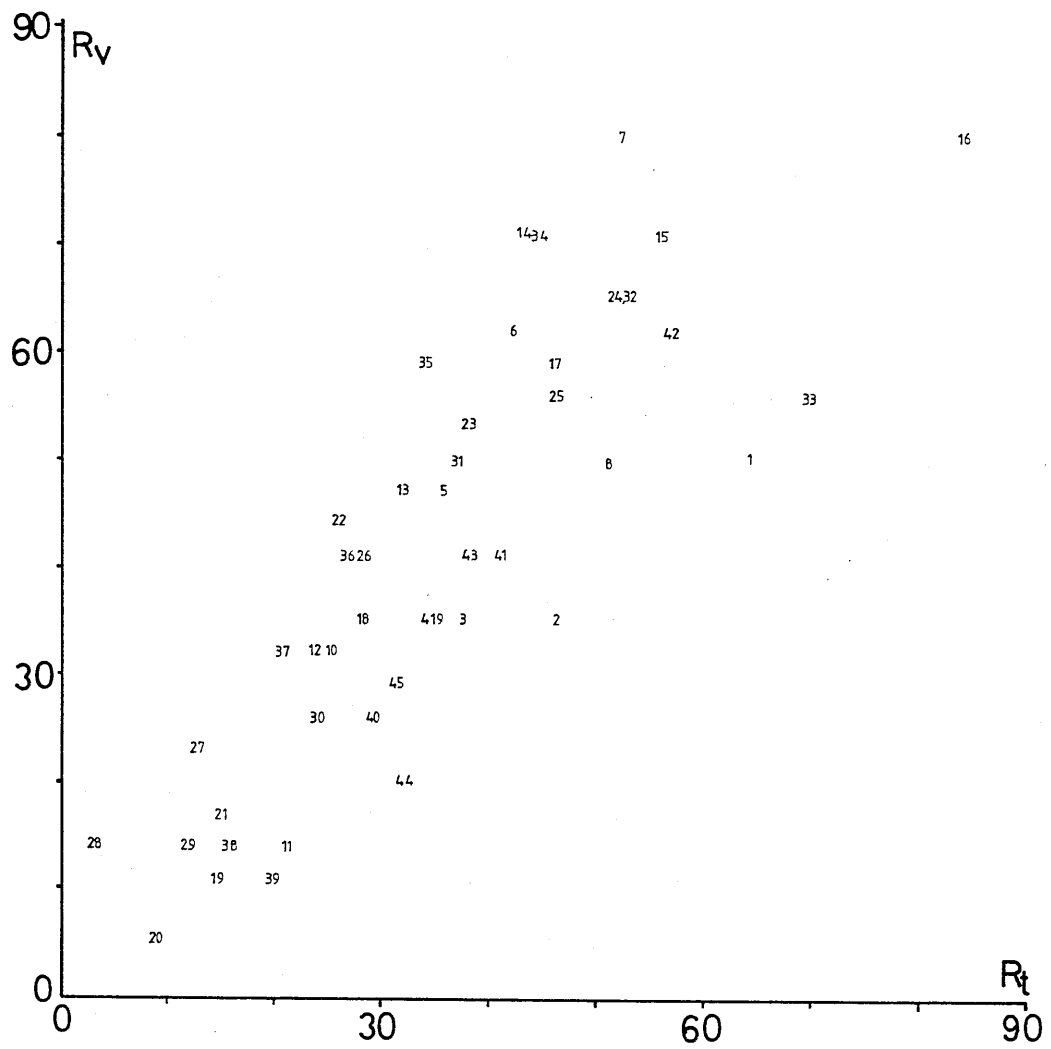
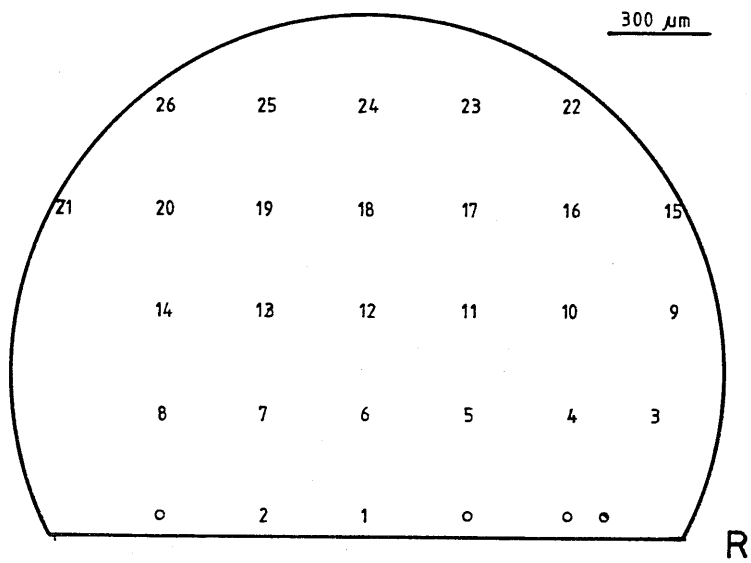
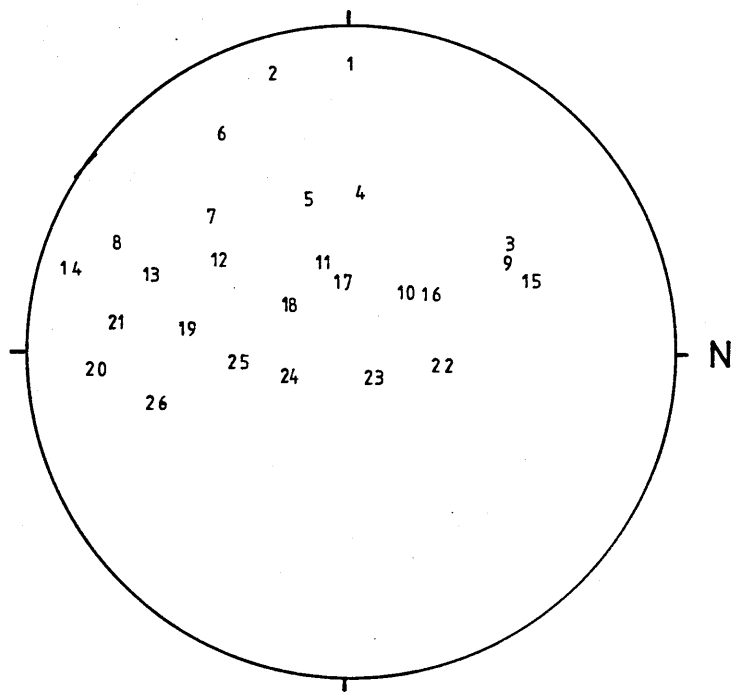


FIGURE 64c



A17D82A

FIGURE 65a

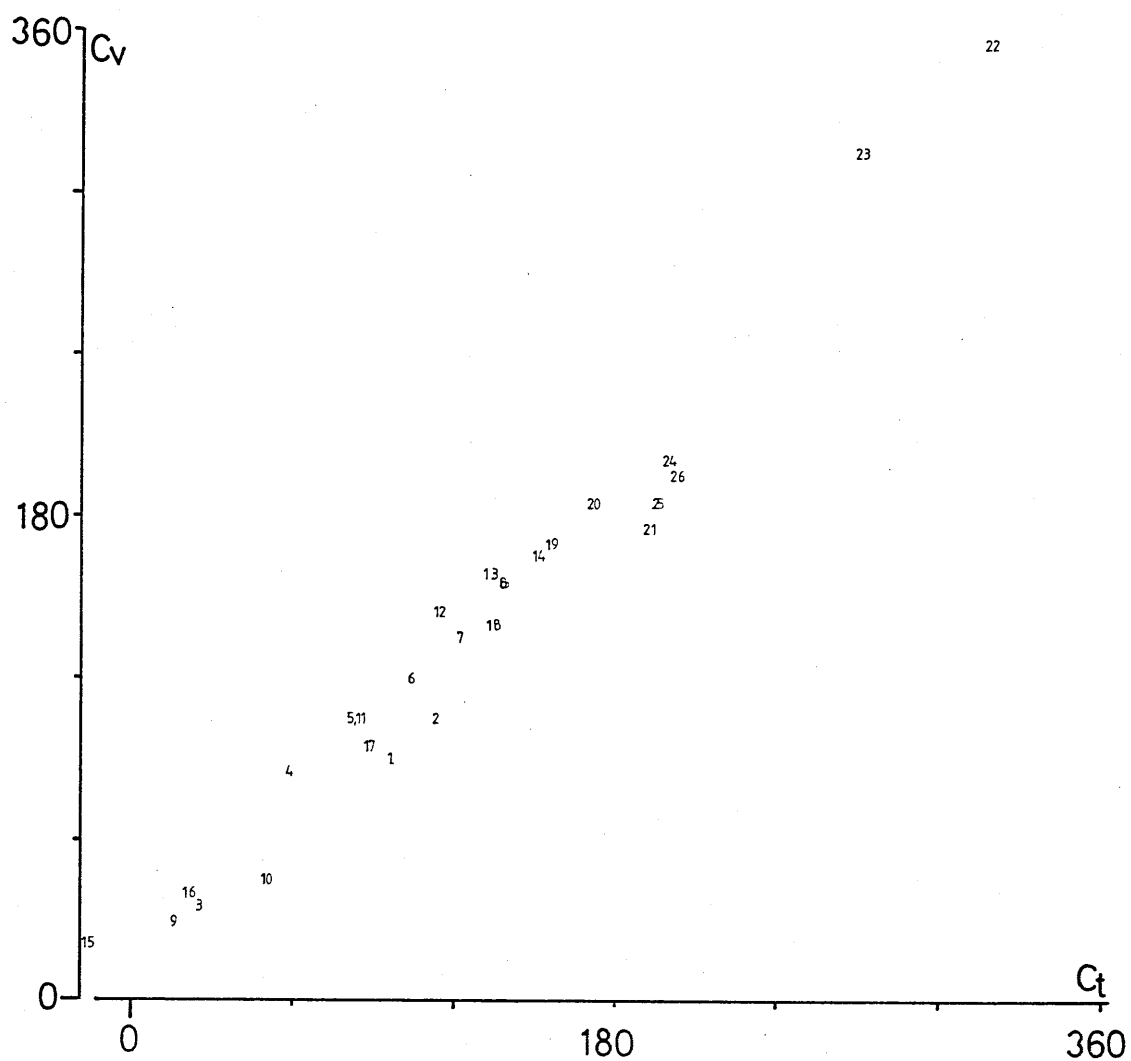


FIGURE 65b



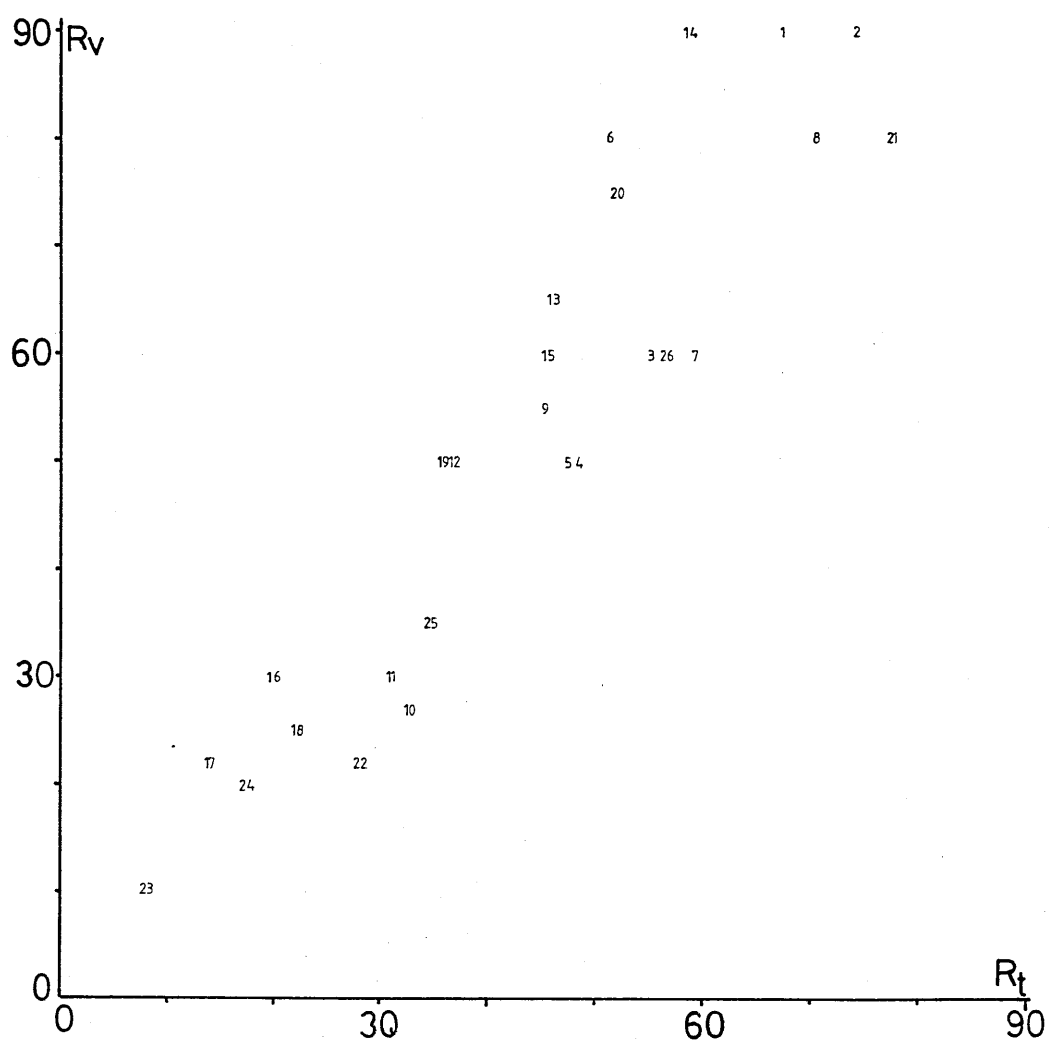
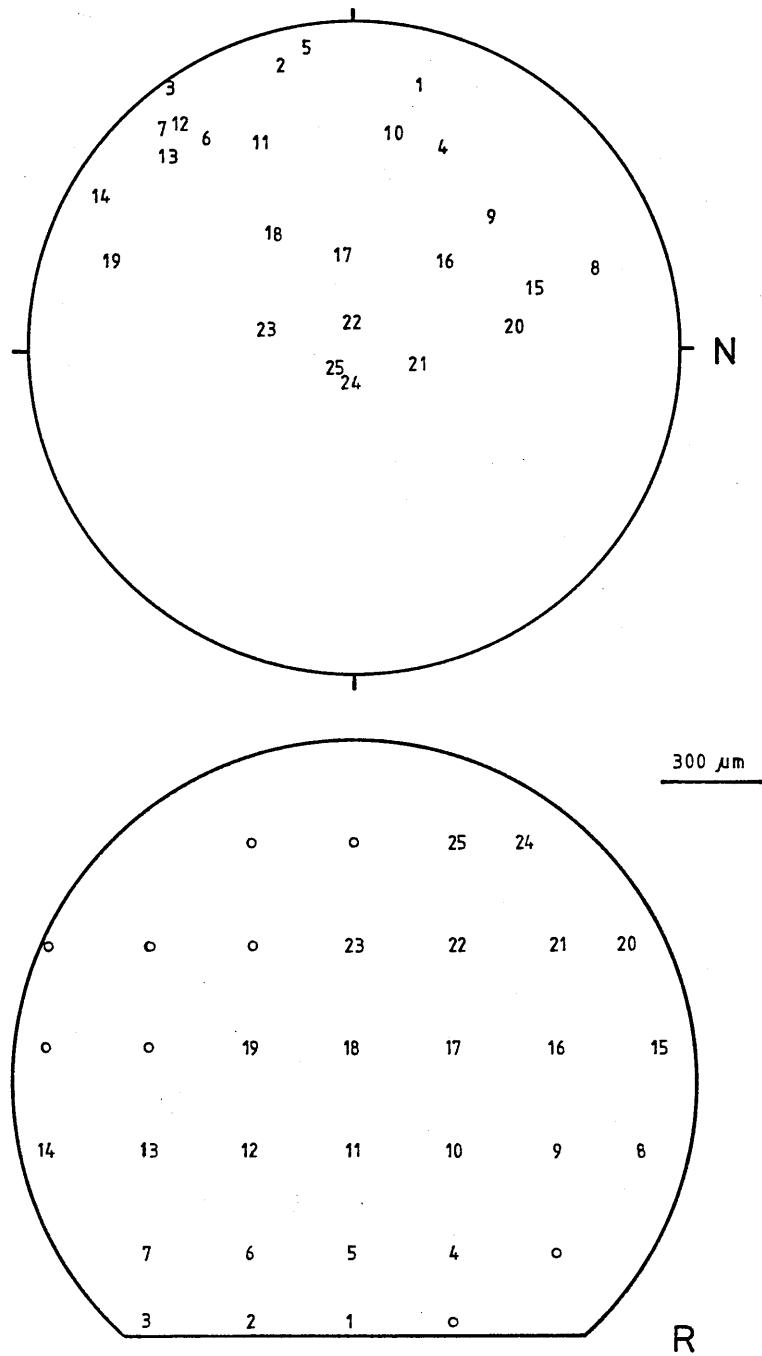


FIGURE 65 c



A17D82B

FIGURE 66a

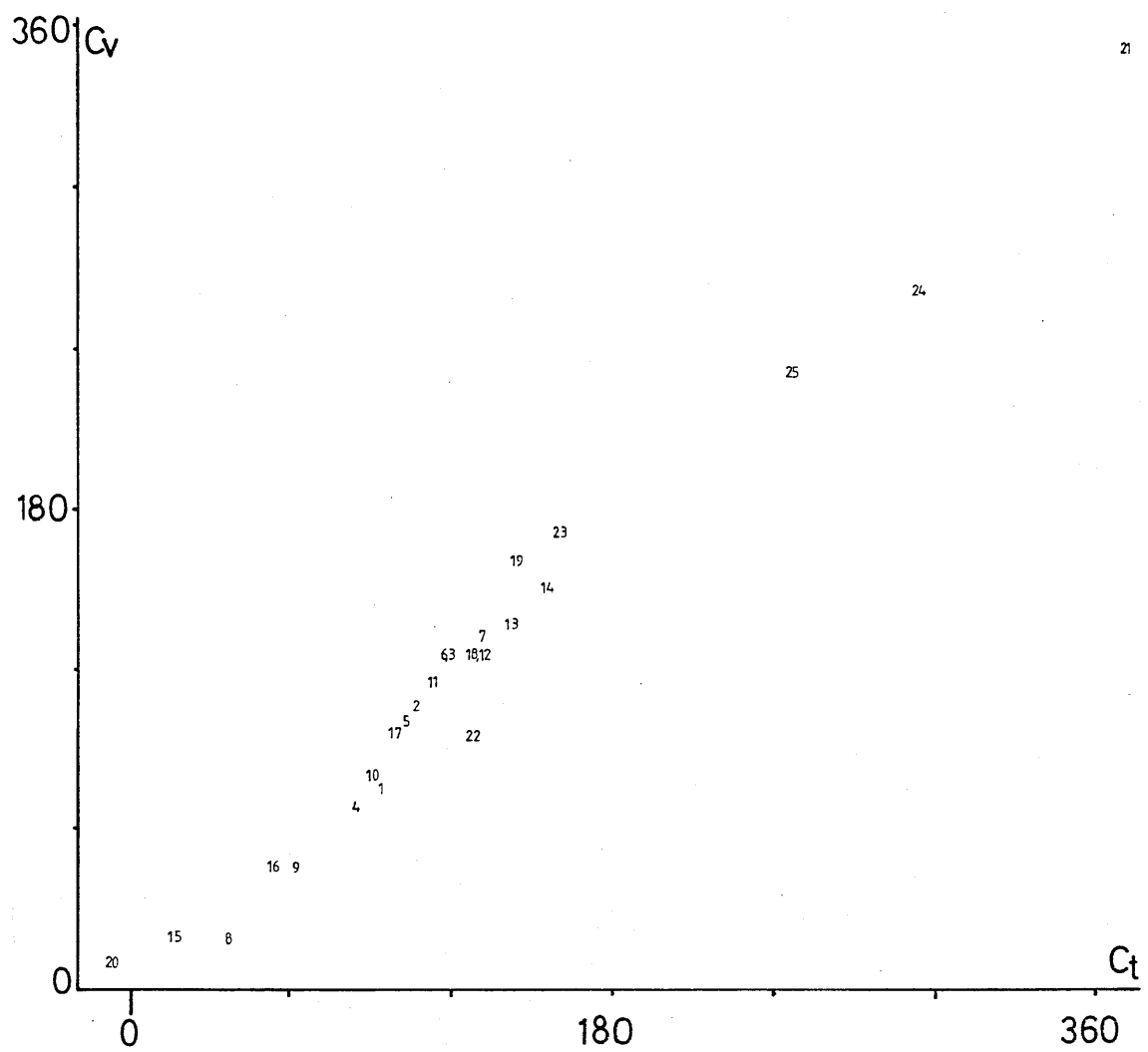


FIGURE 66 b

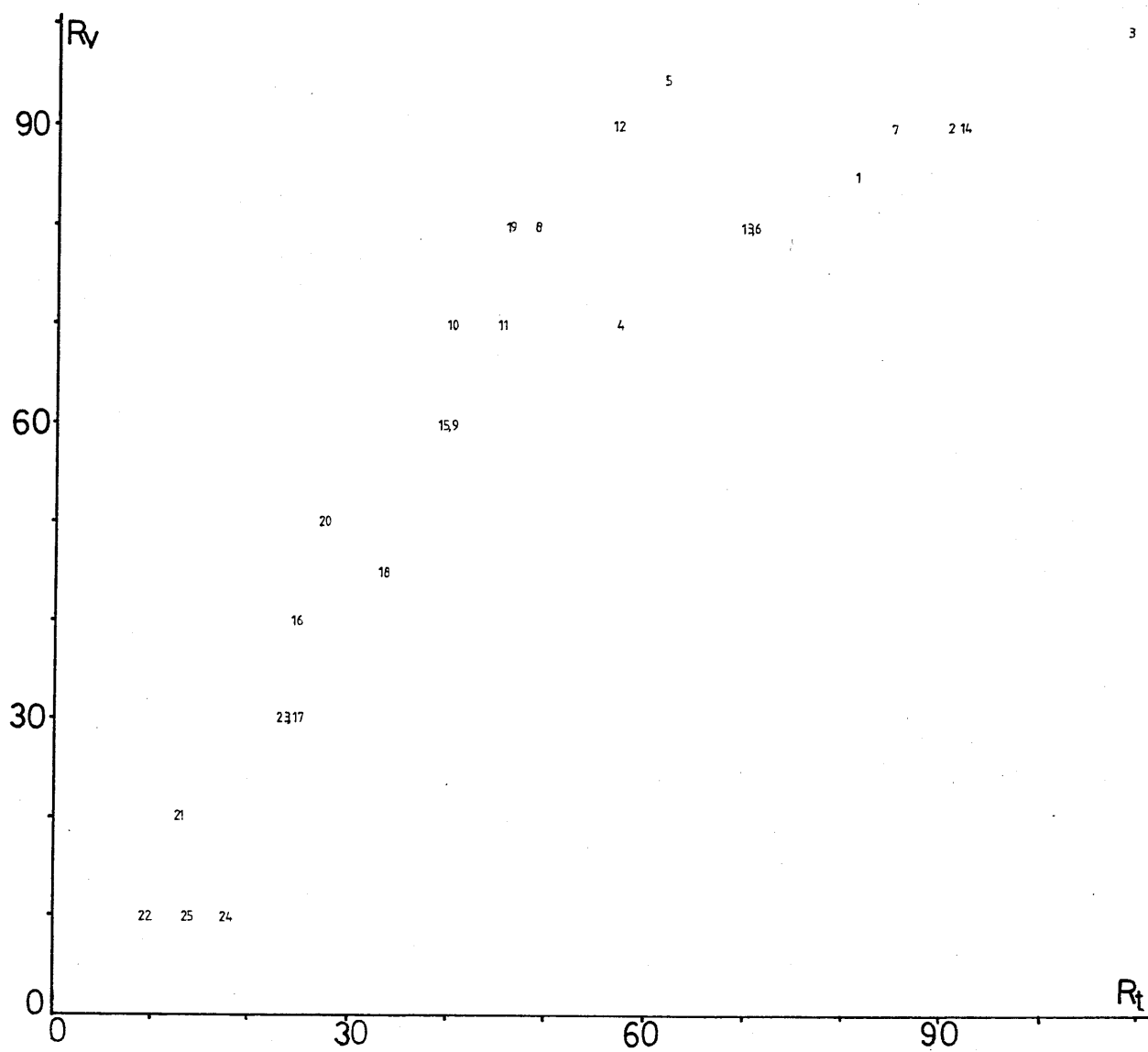


FIGURE 66 c

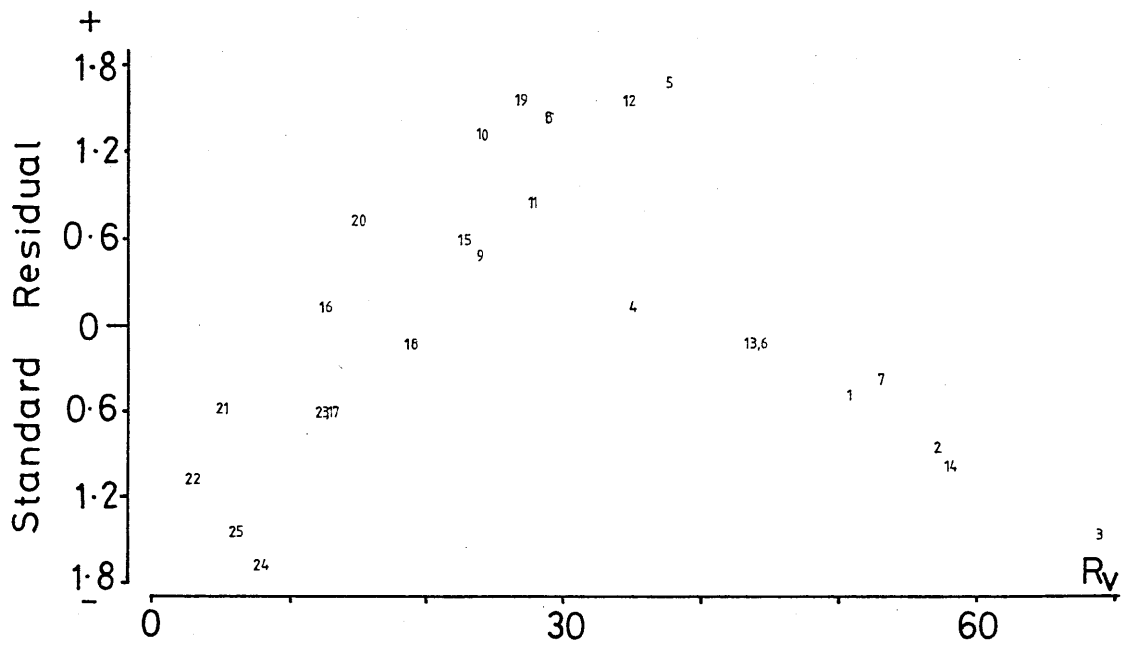
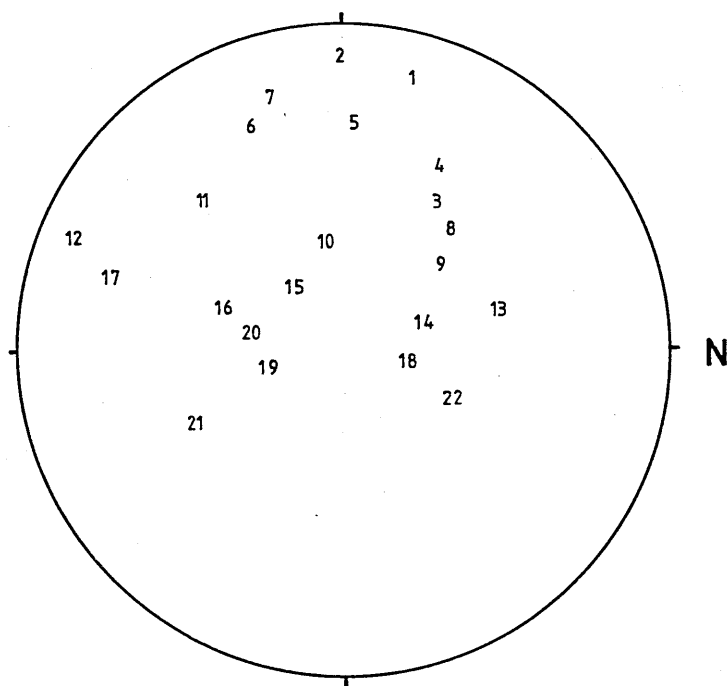
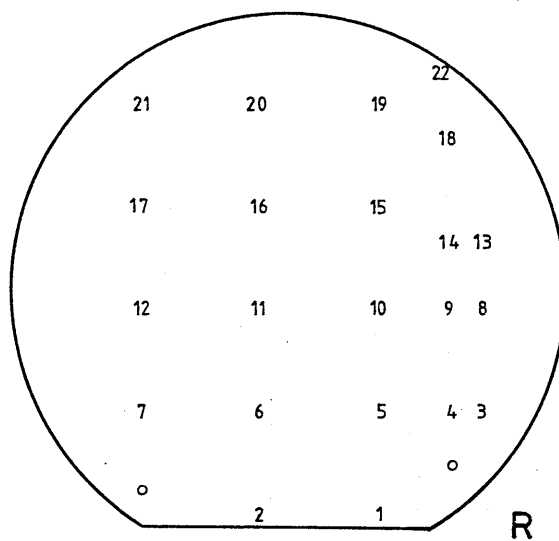


FIGURE 66 d



300  $\mu$ m



A 20D129

FIGURE 67a

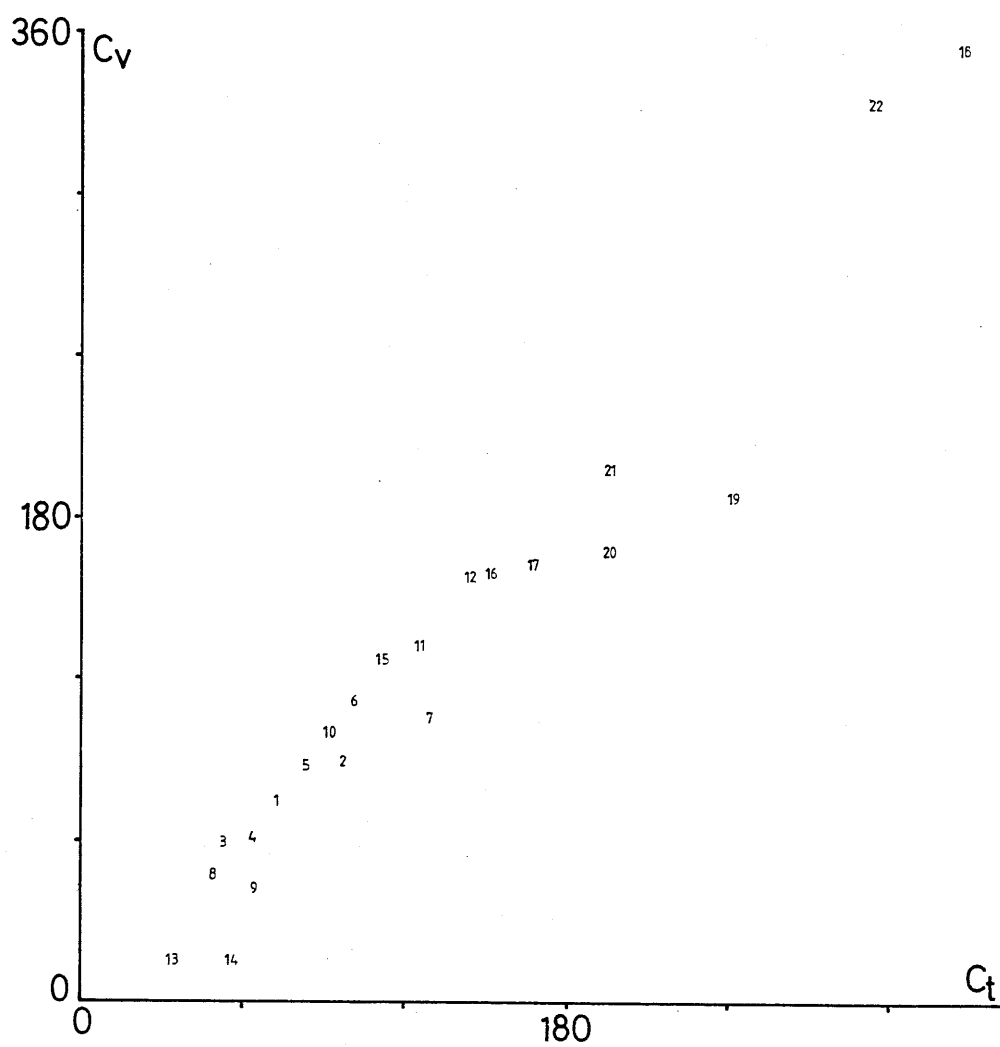


FIGURE 67 b

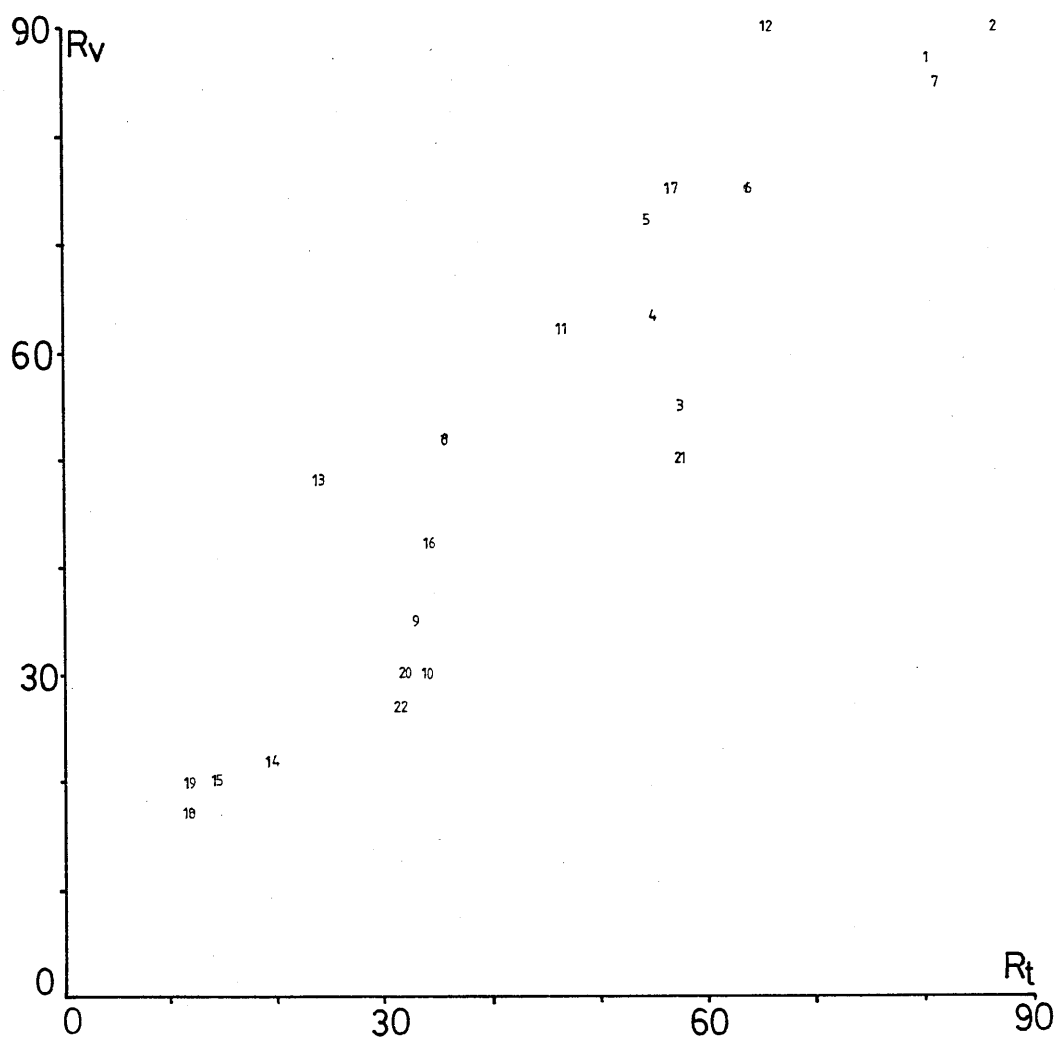
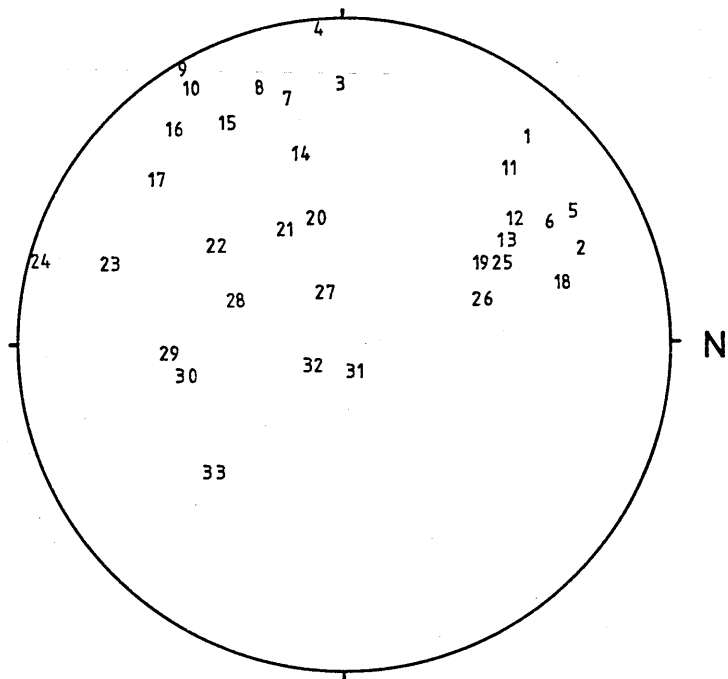
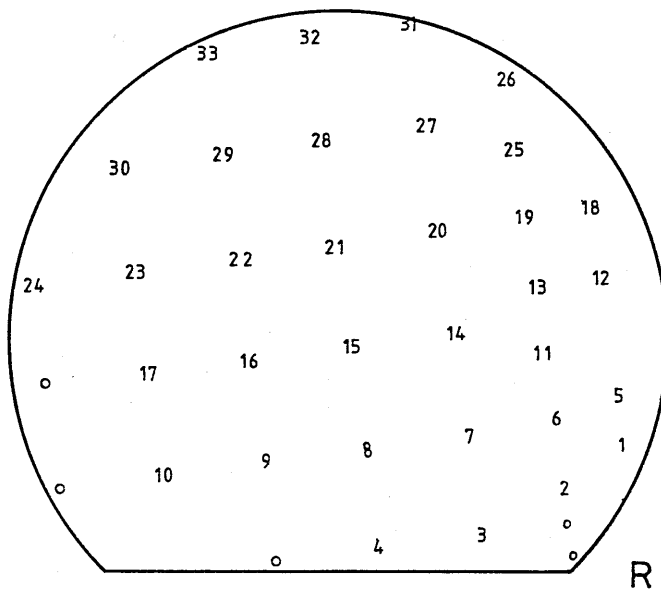


FIGURE 67c





300  $\mu\text{m}$



A 33 D134

FIGURE 68a

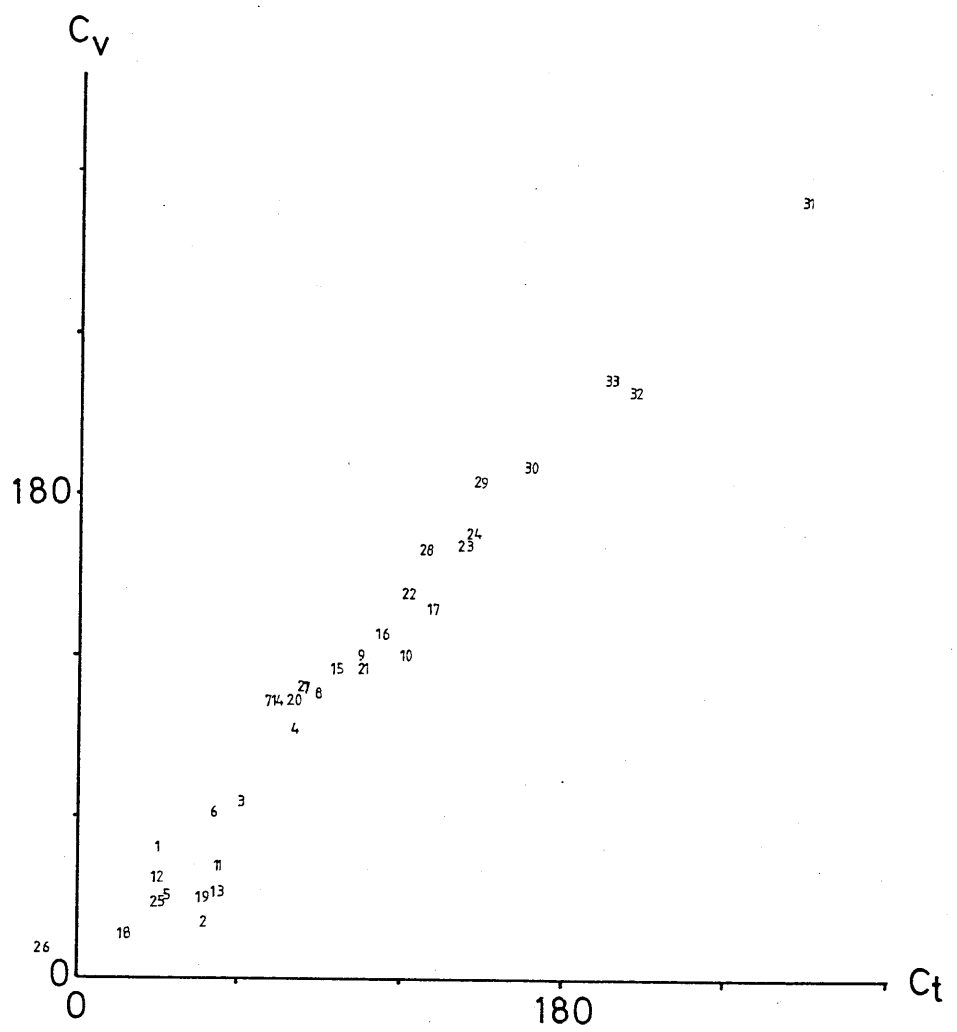


FIGURE 68 b

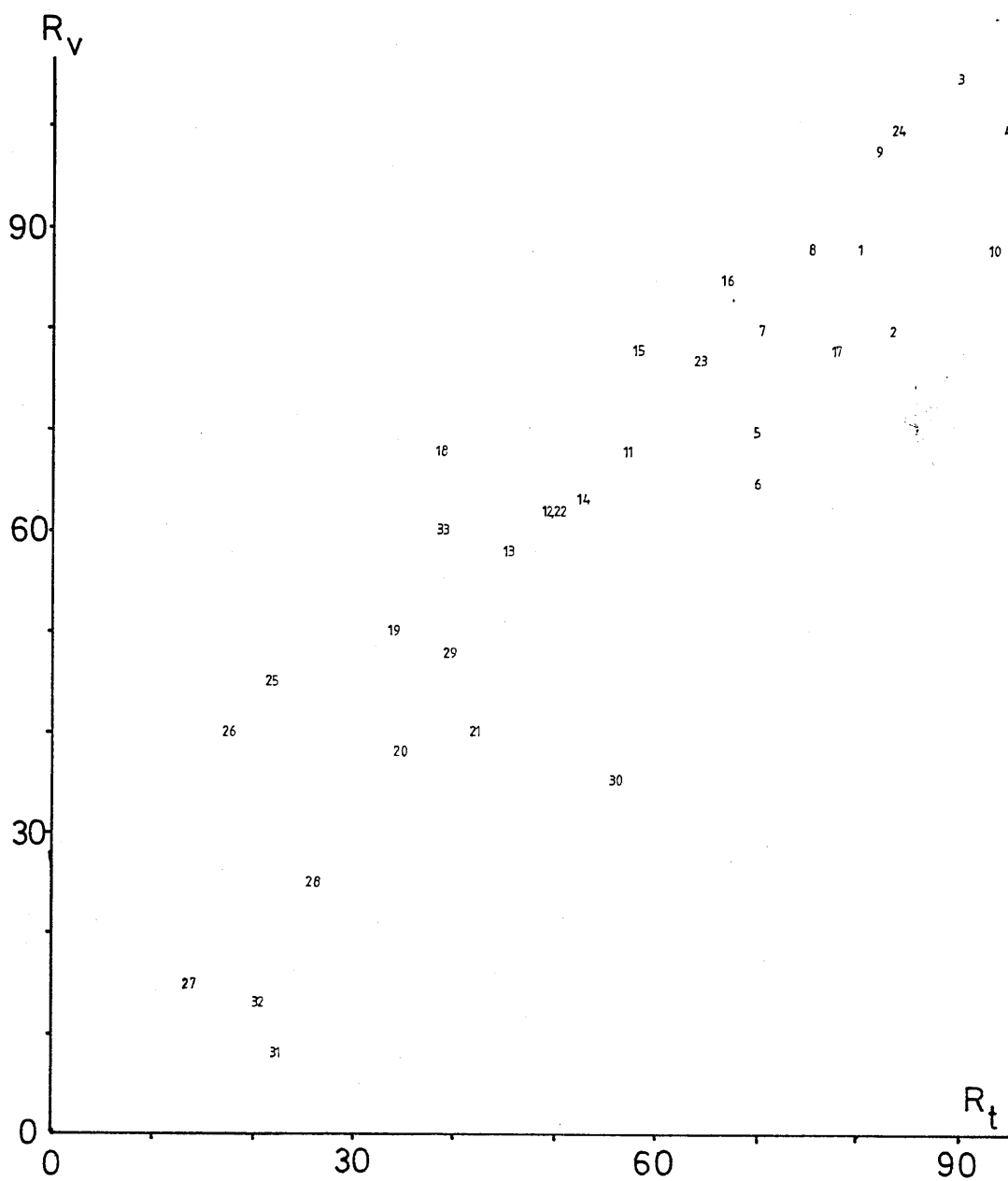


FIGURE 68 c

Standard  
residual

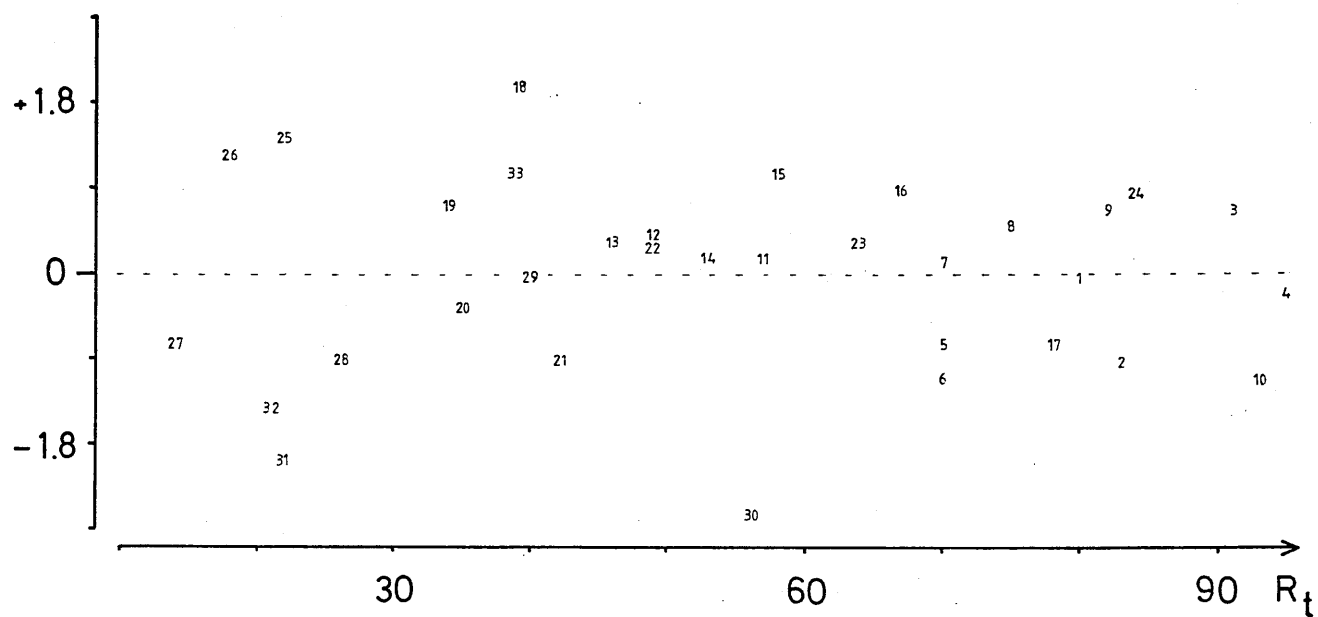


FIGURE 68d

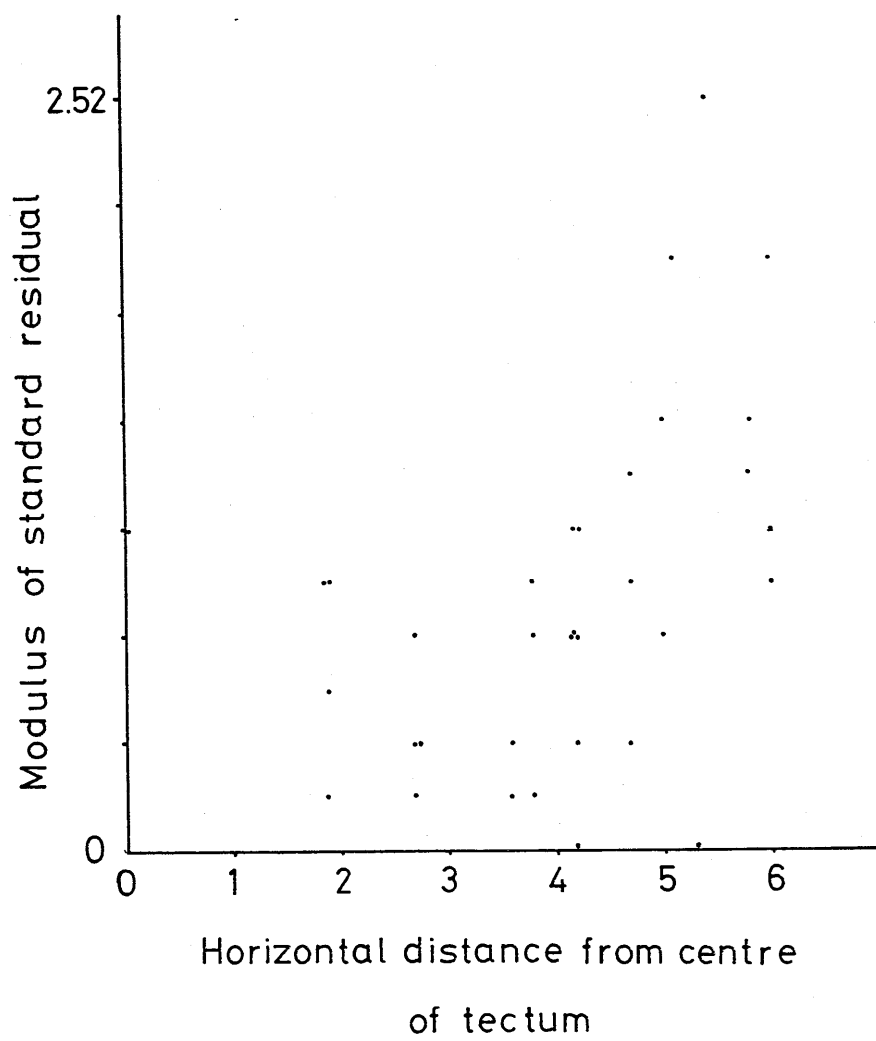
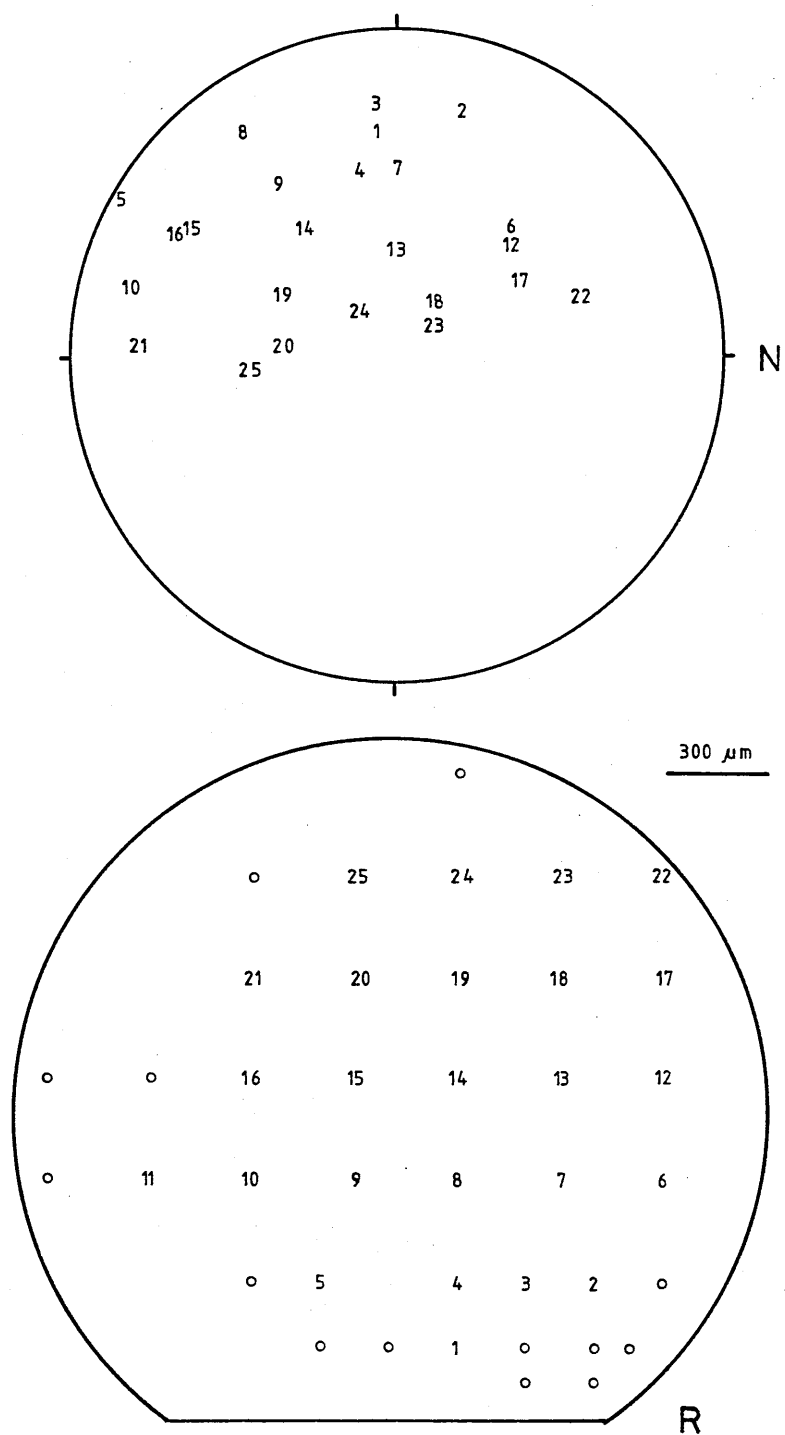


FIGURE 68e



A 33D158

FIGURE 69a

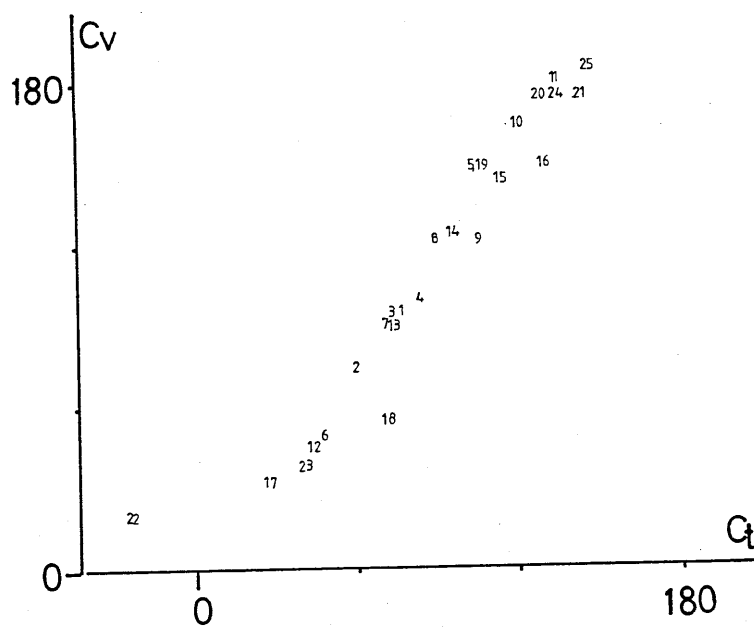


FIGURE 69b

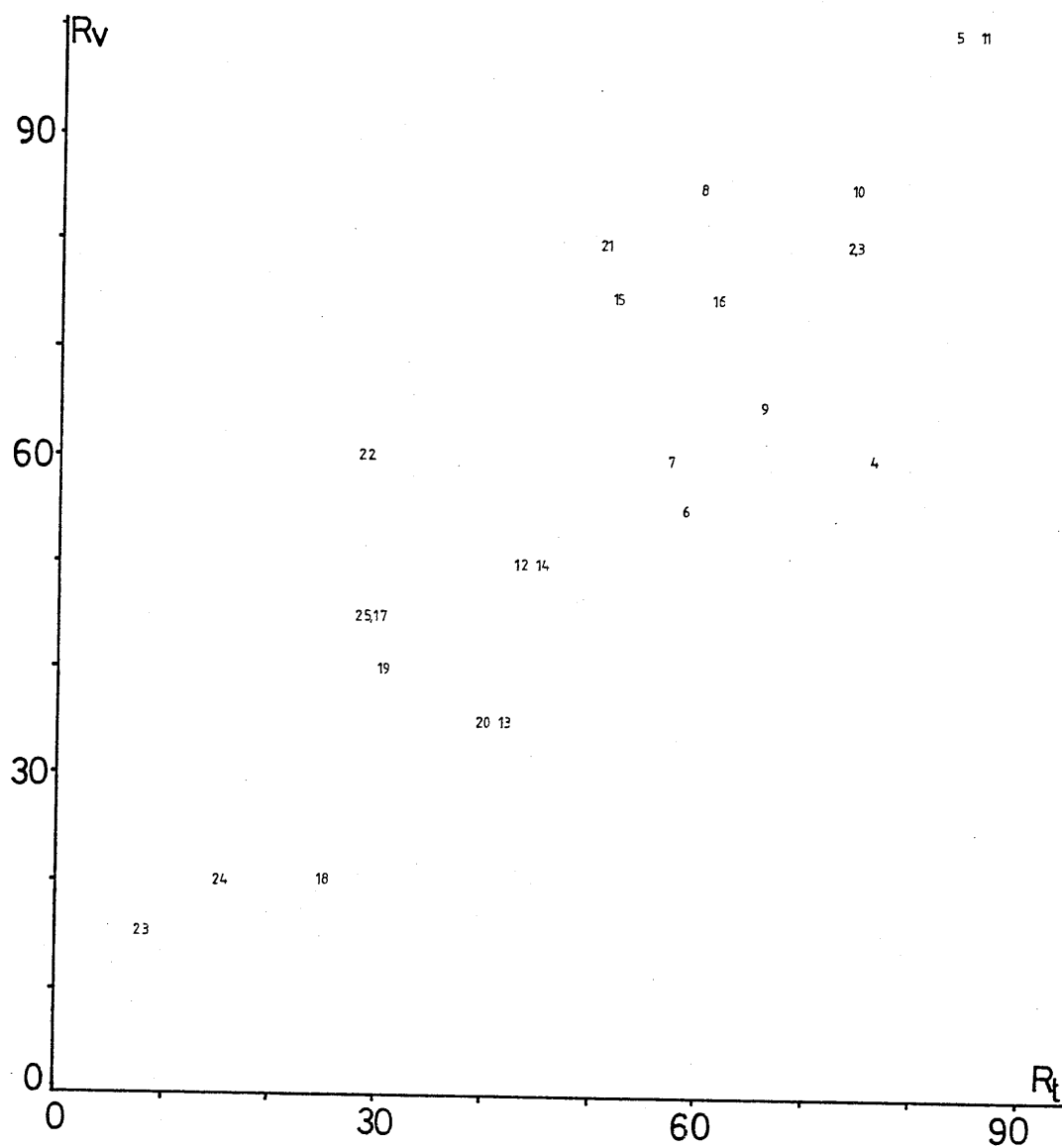
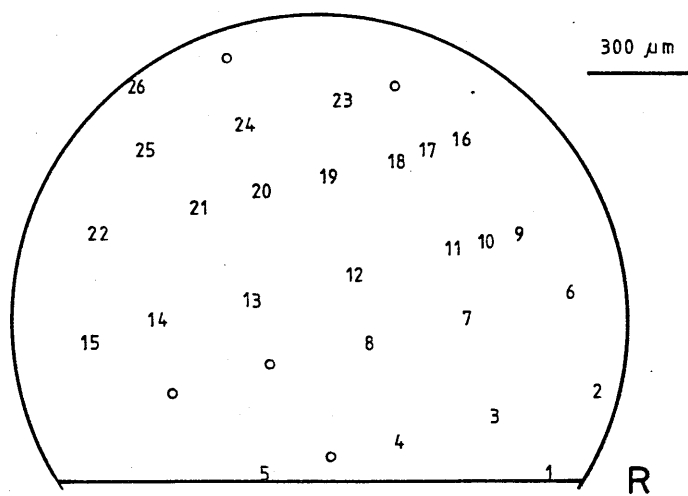
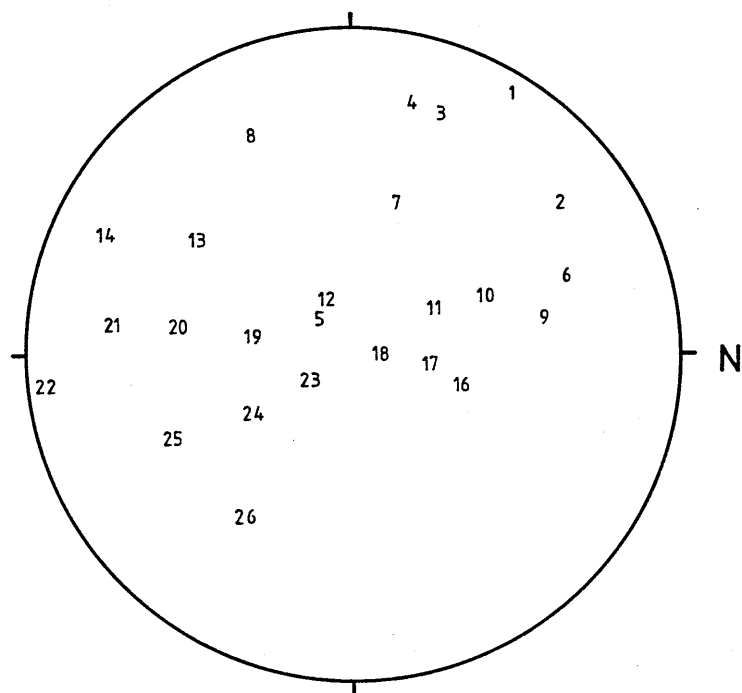


FIGURE 69c





A 17 D 42

FIGURE 70a

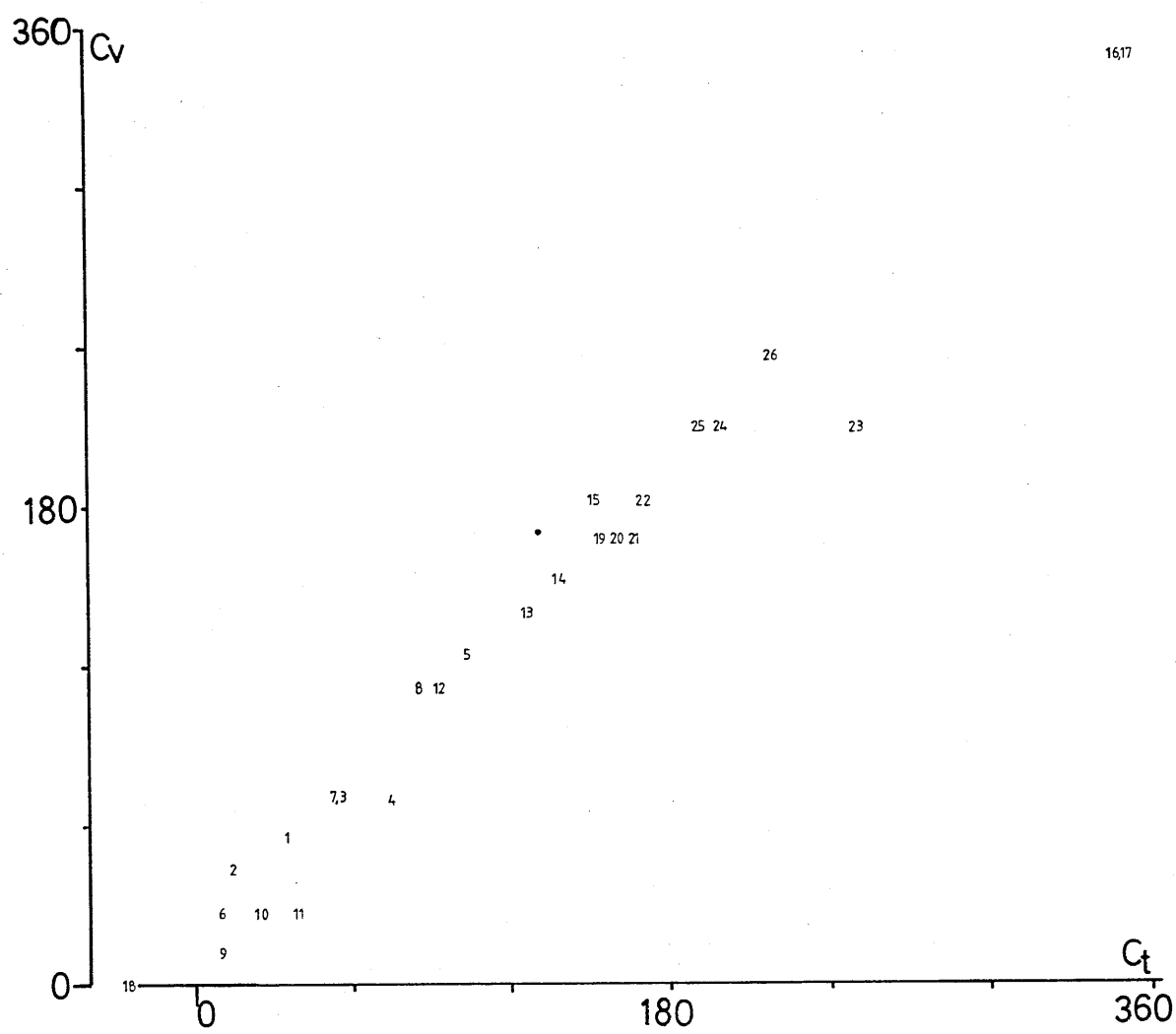


FIGURE 70b

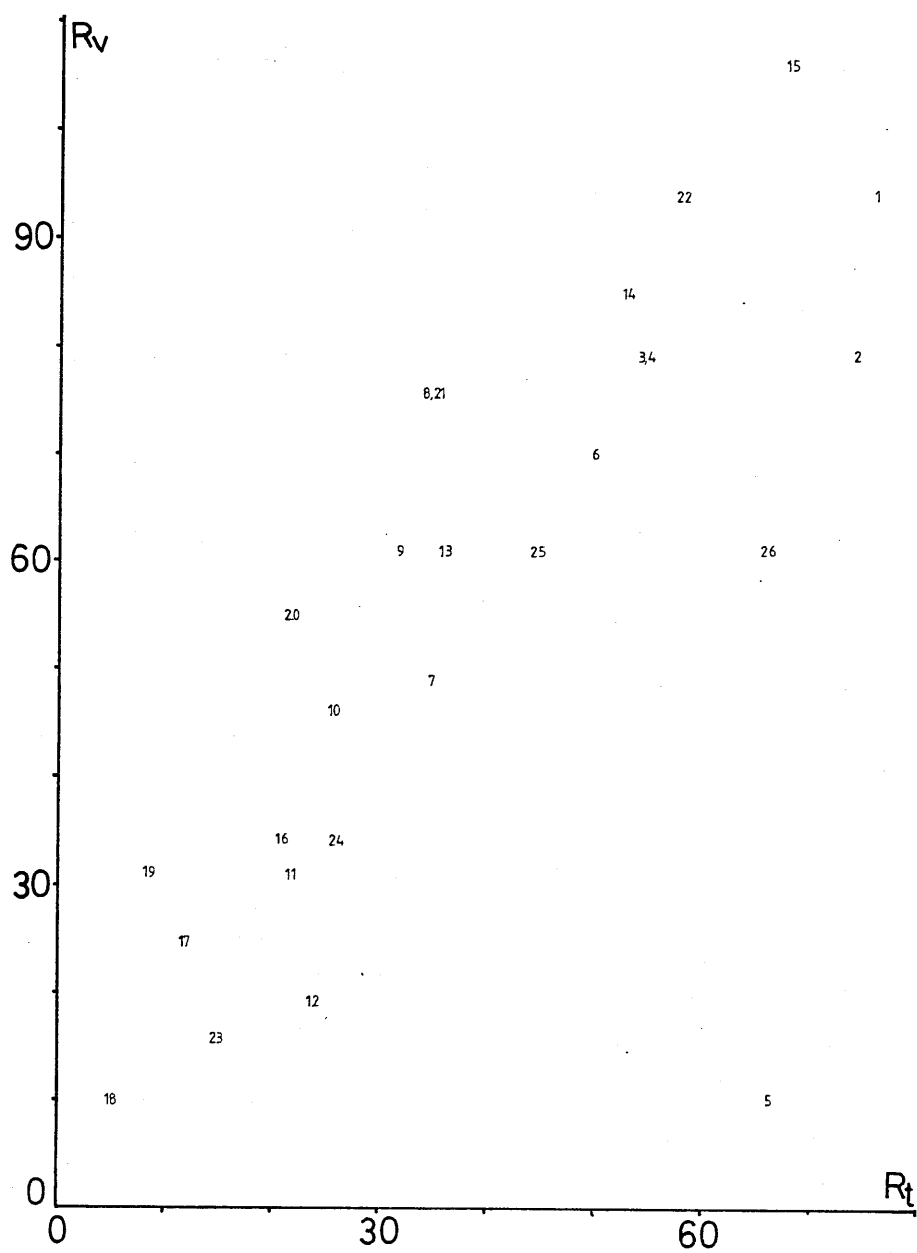


FIGURE 70 c

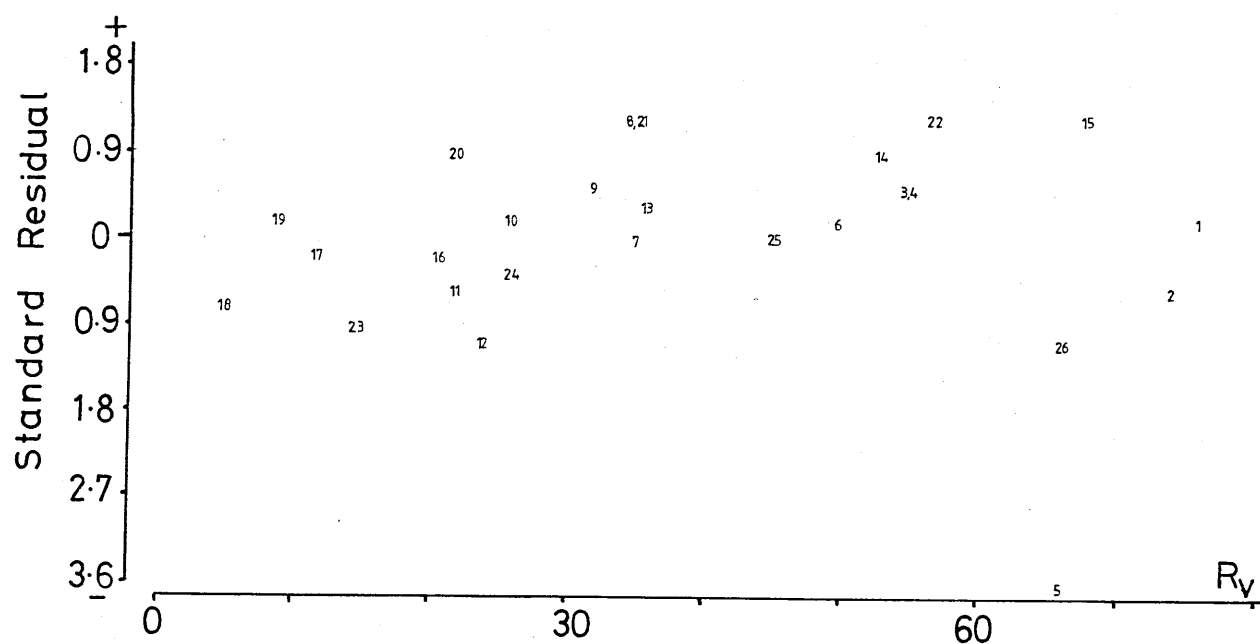
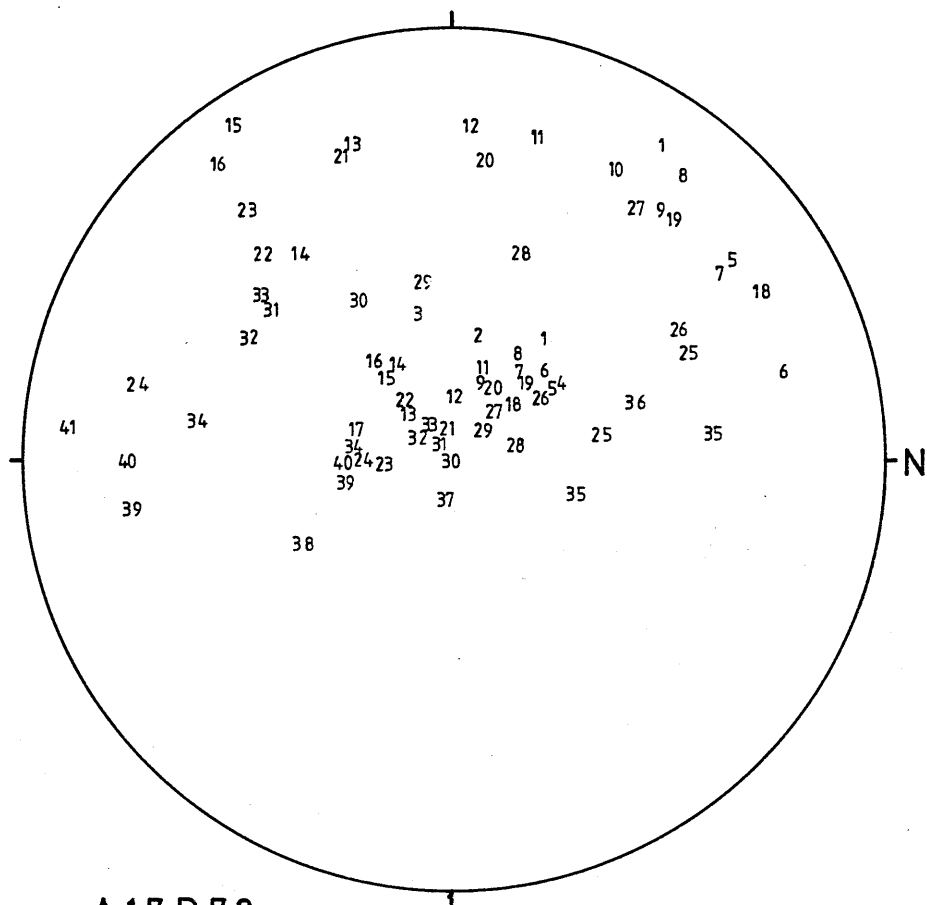


FIGURE 70 d



A17D72

300  $\mu$ m

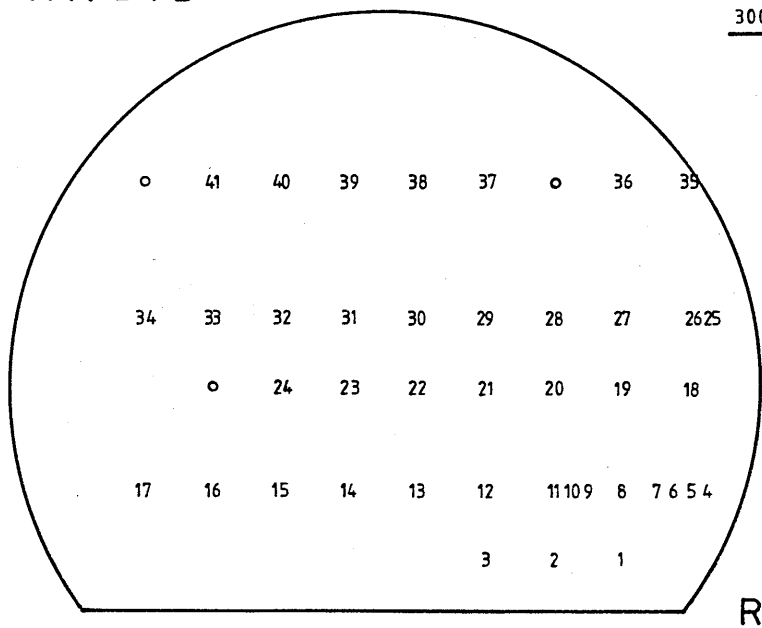


FIGURE 71a

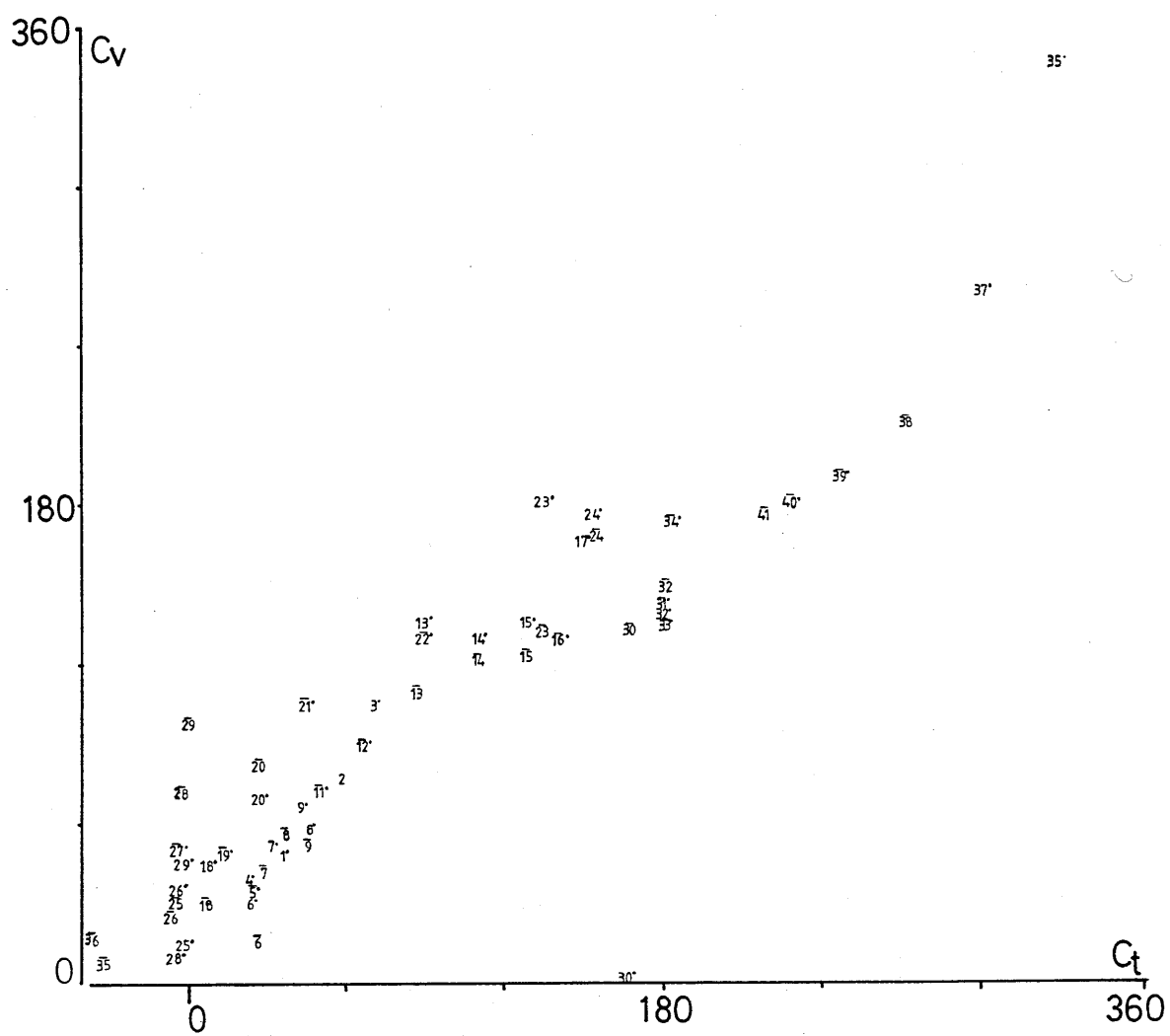


FIGURE 71b

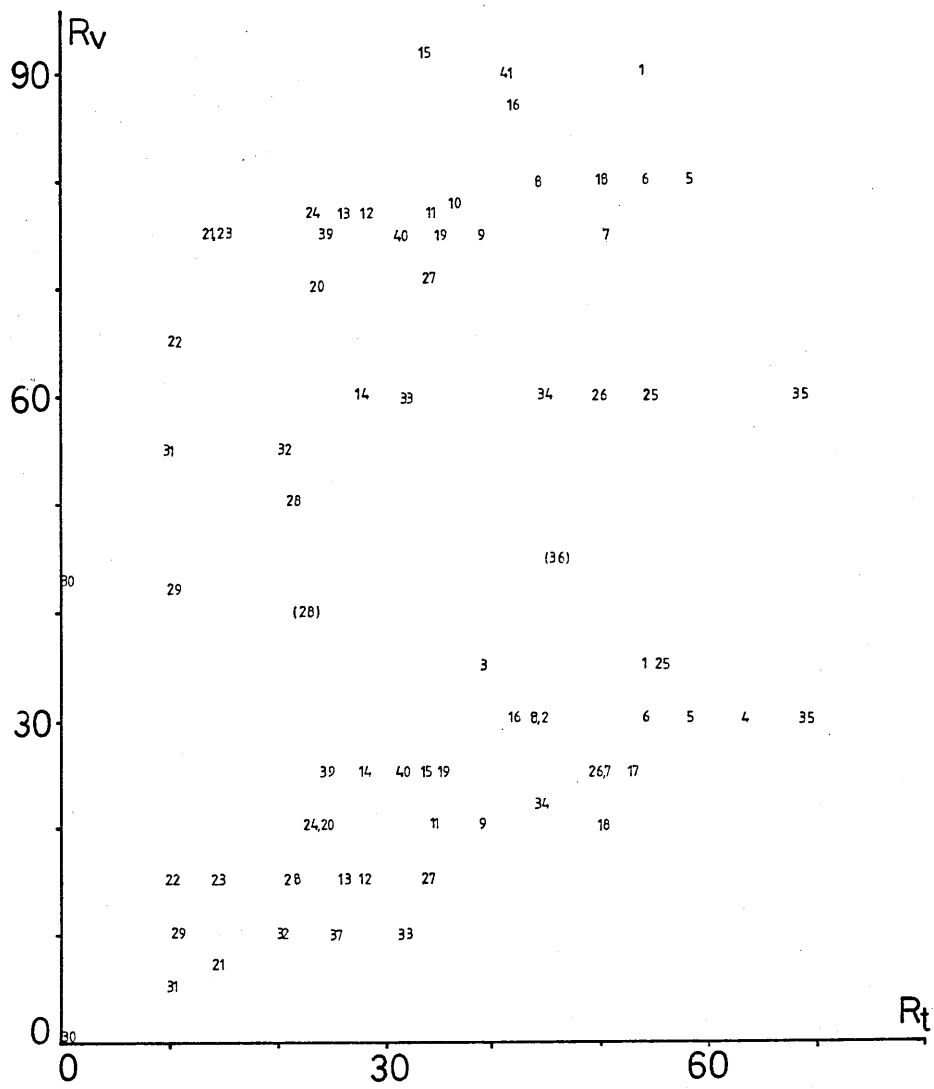


FIGURE 71c

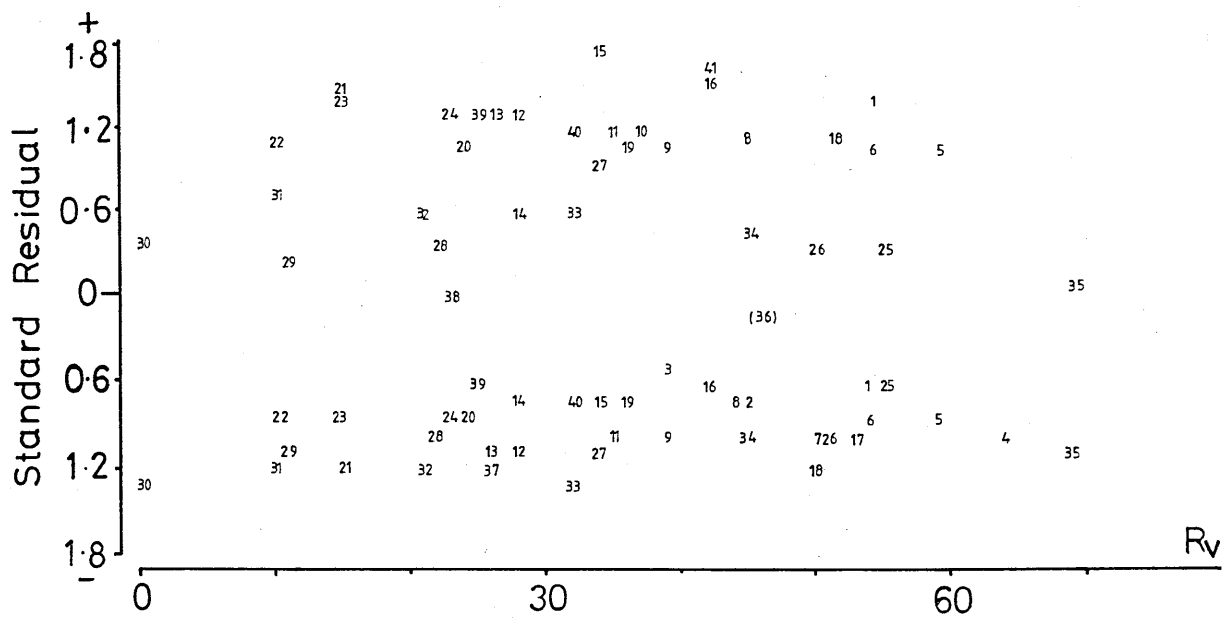


FIGURE 71d



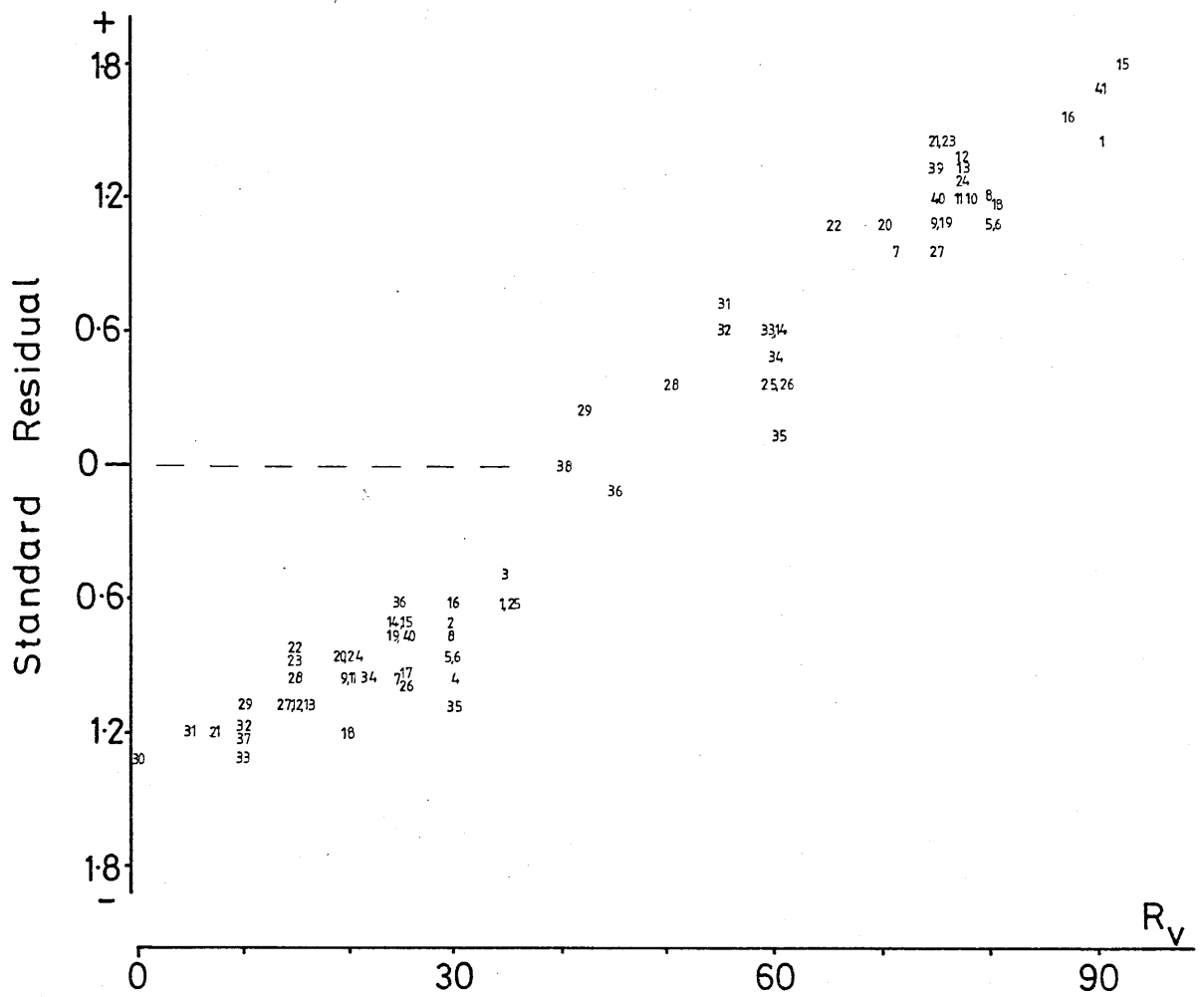
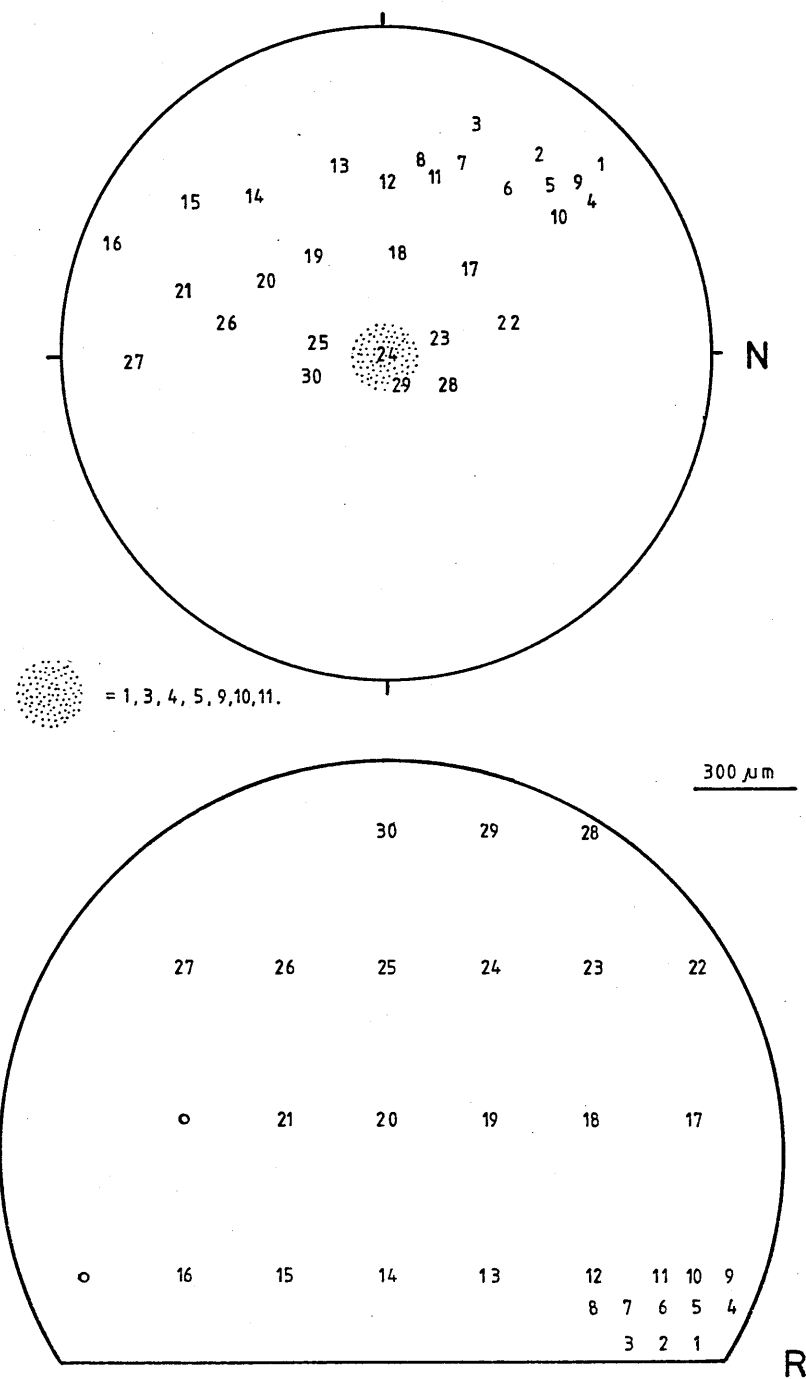


FIGURE 71e



A17D8I

FIGURE 72a

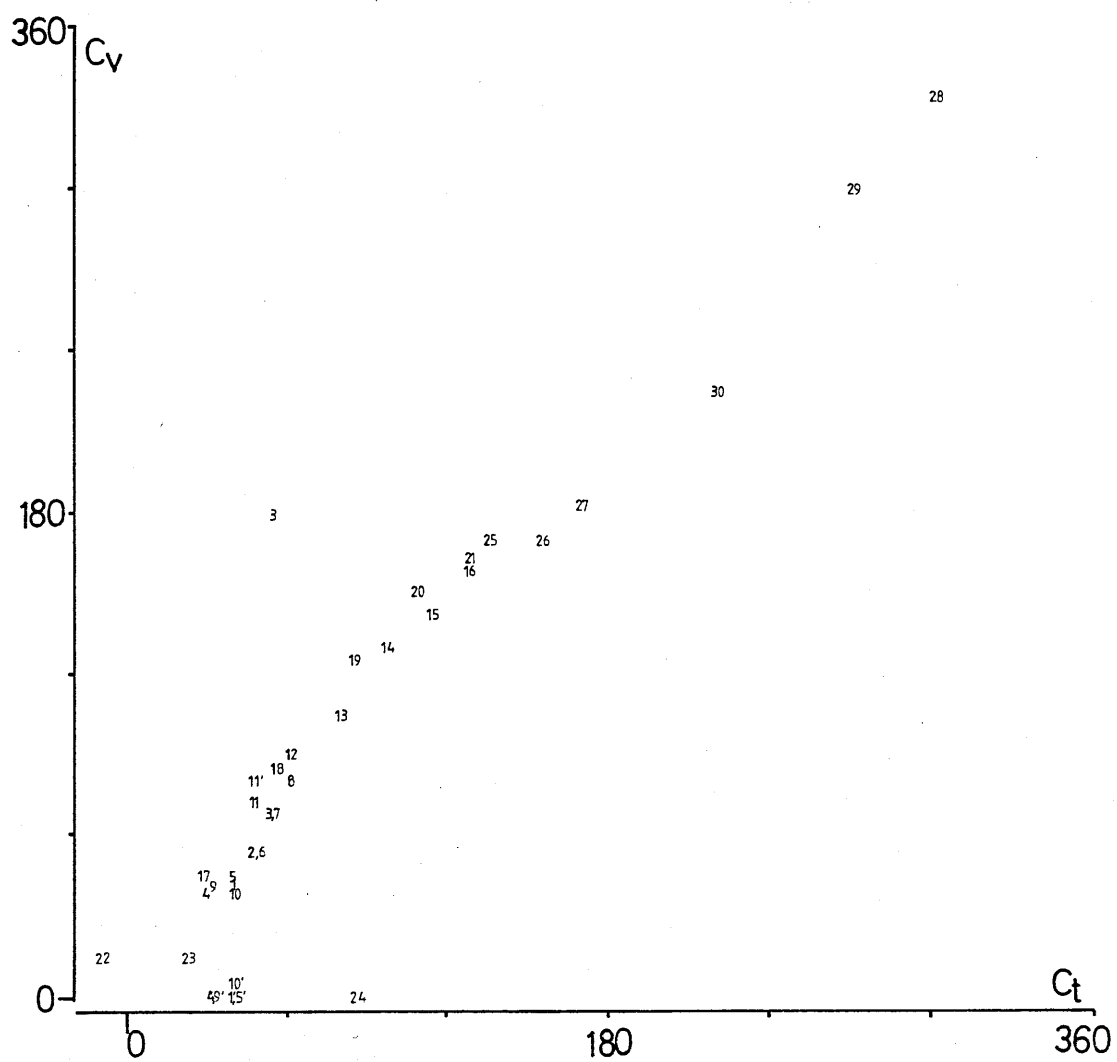


FIGURE 72 b

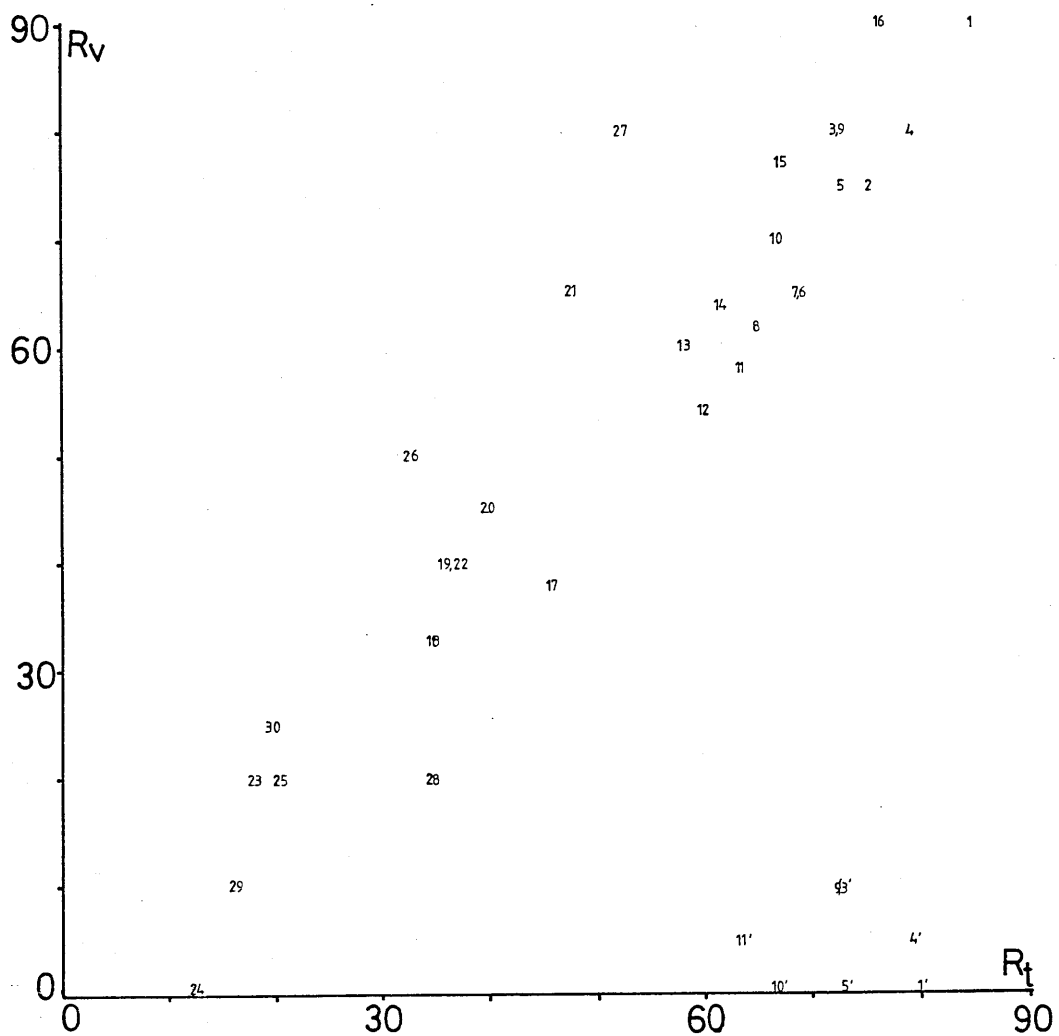
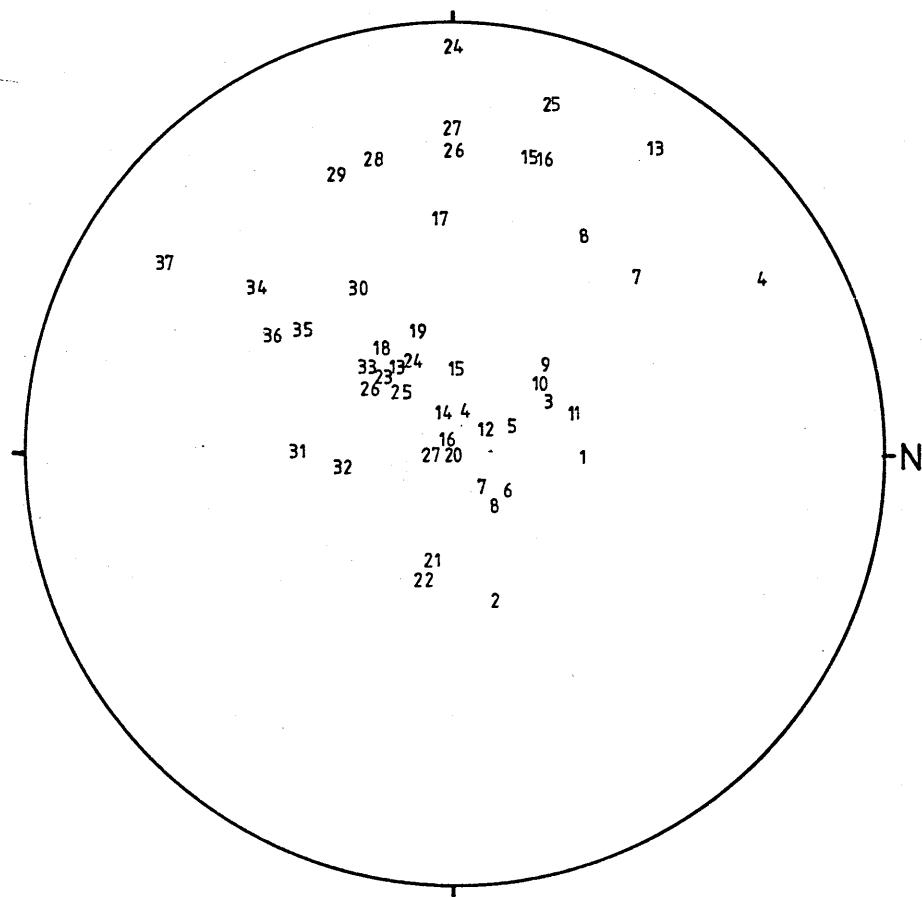
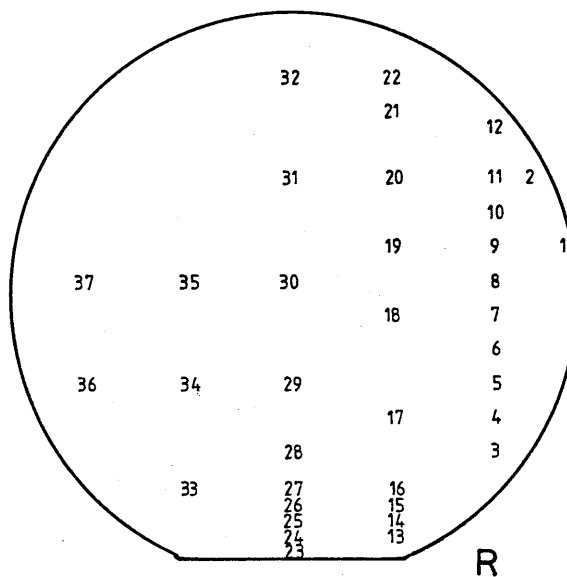


FIGURE 72c



A 20 D 30

300  $\mu$ m



R

FIGURE 73a

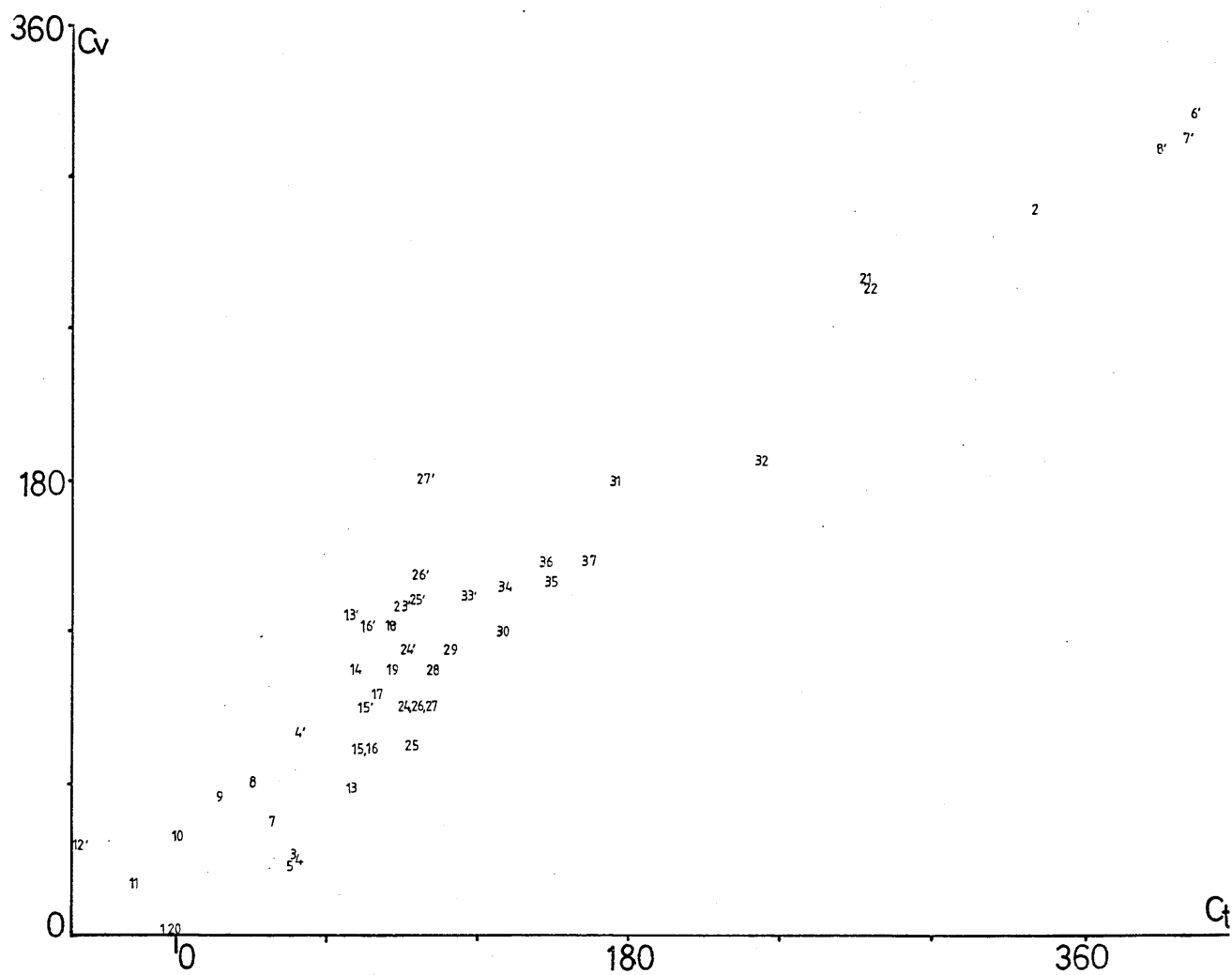


FIGURE 73b

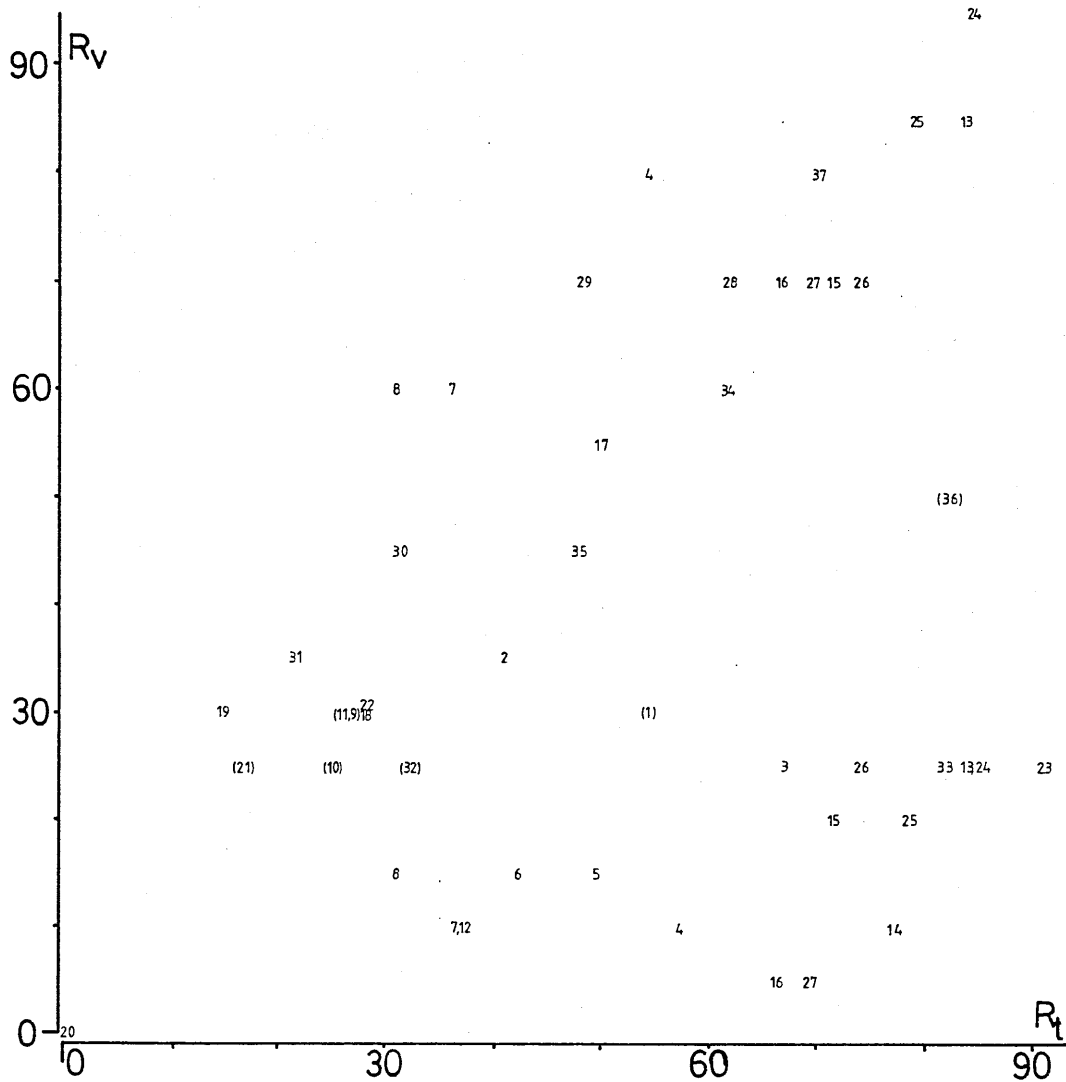
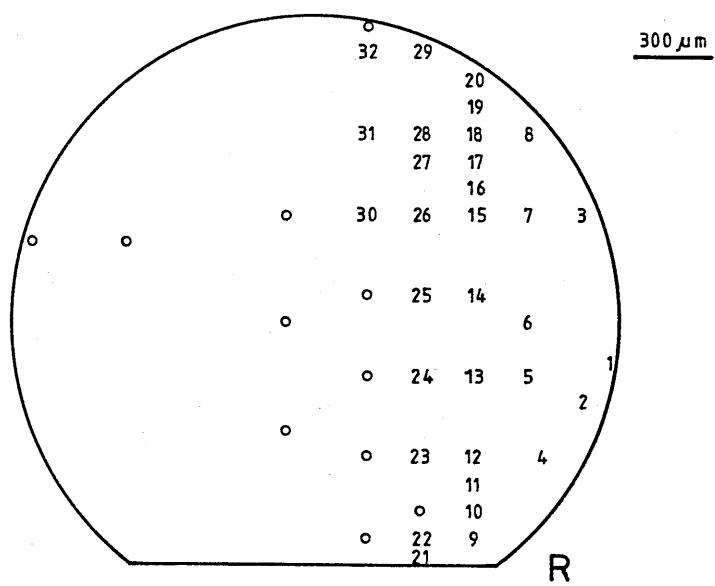
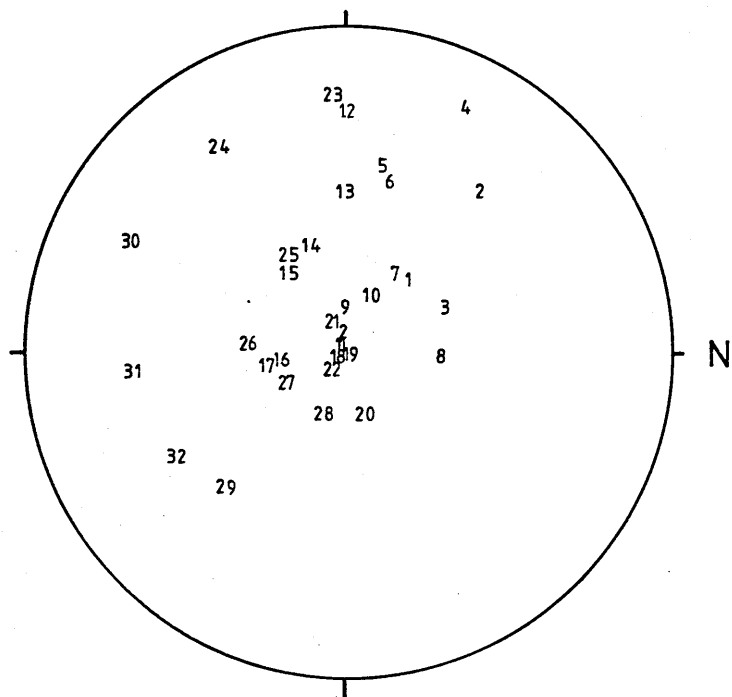


FIGURE 73c



A 20D31

FIGURE 74a



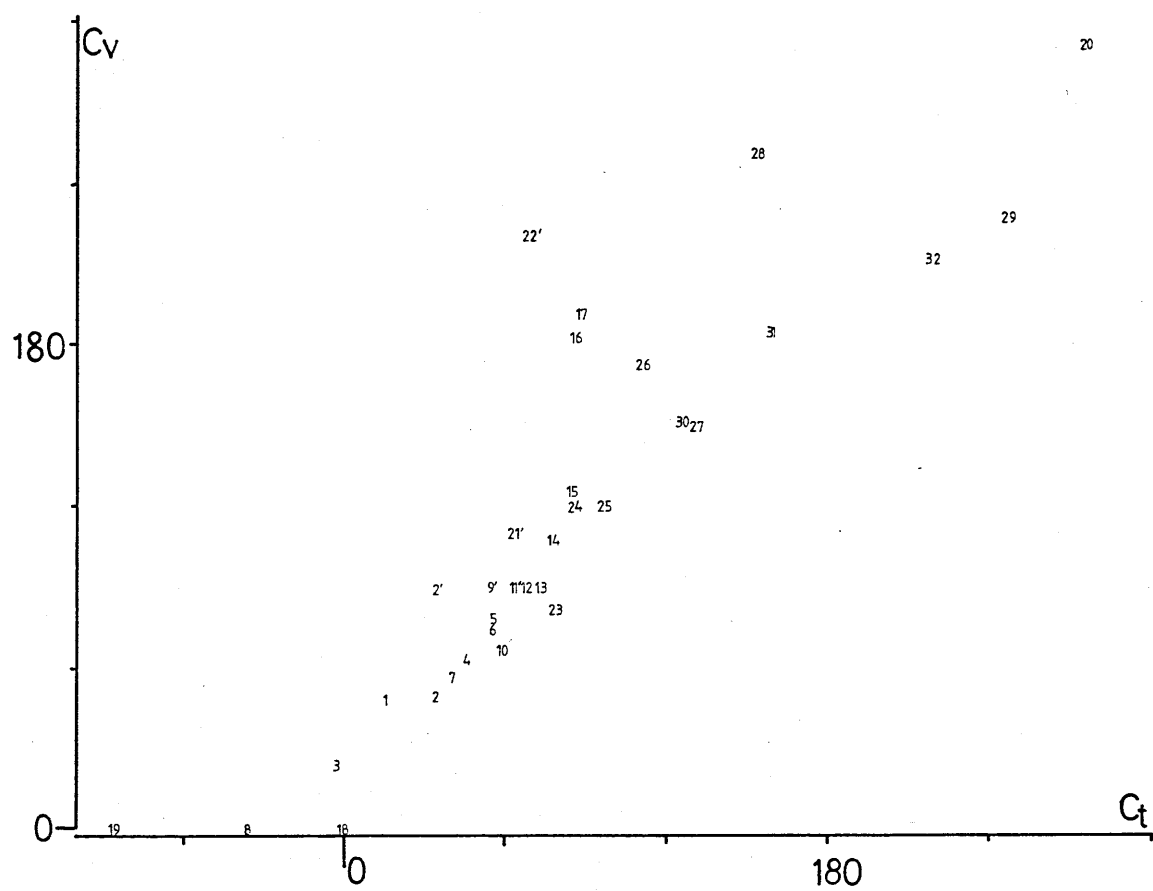


FIGURE 74 b

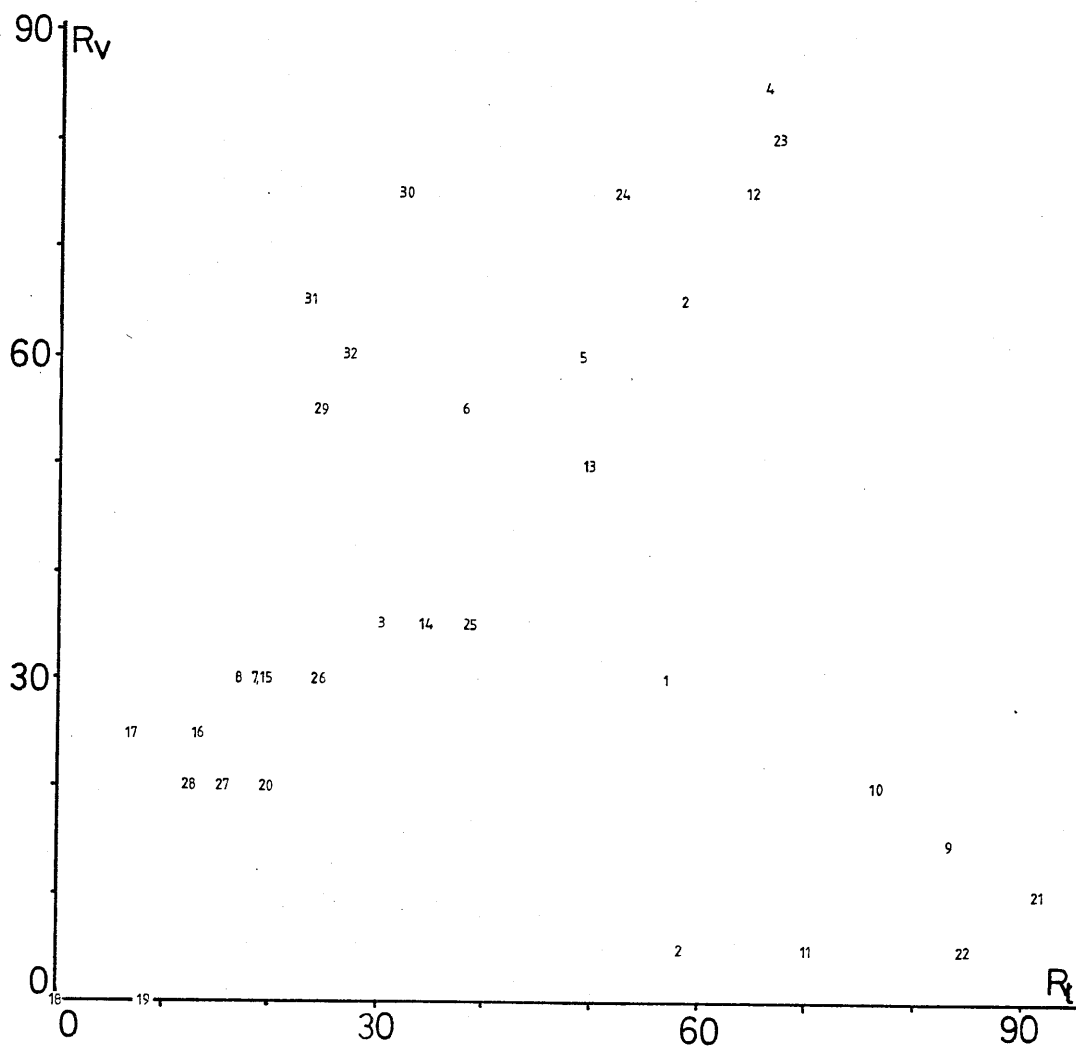
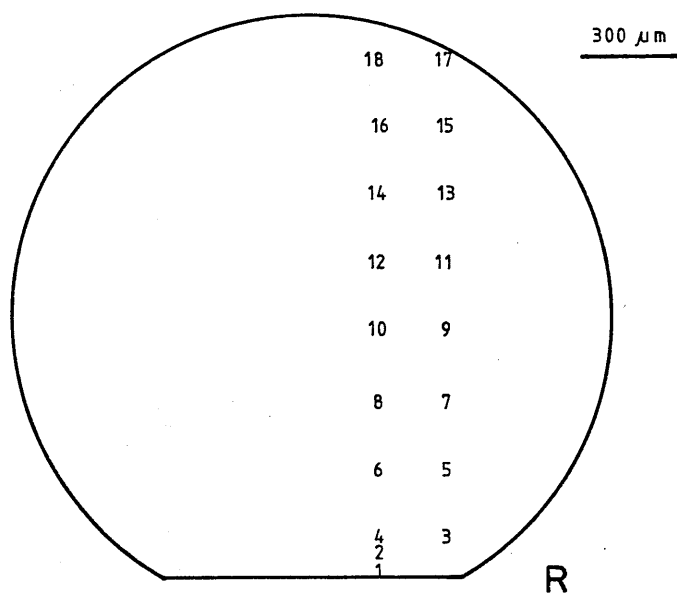
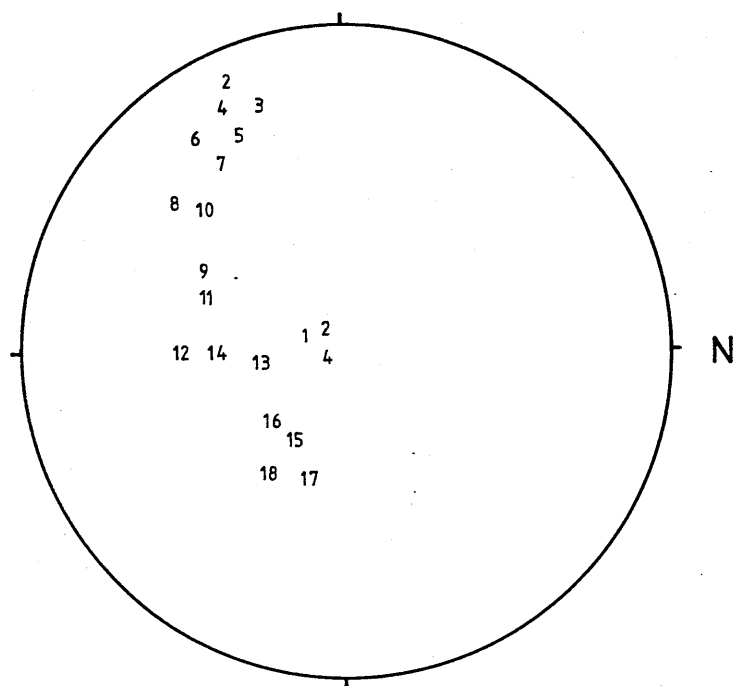


FIGURE 74c



A 20 D119

FIGURE 75 a

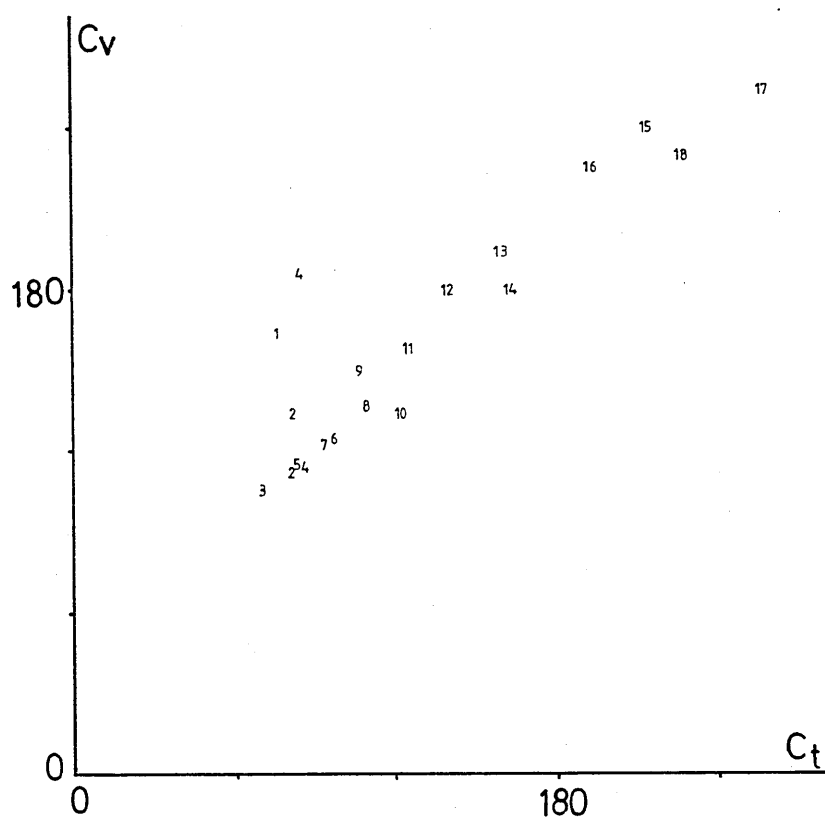


FIGURE 75 b

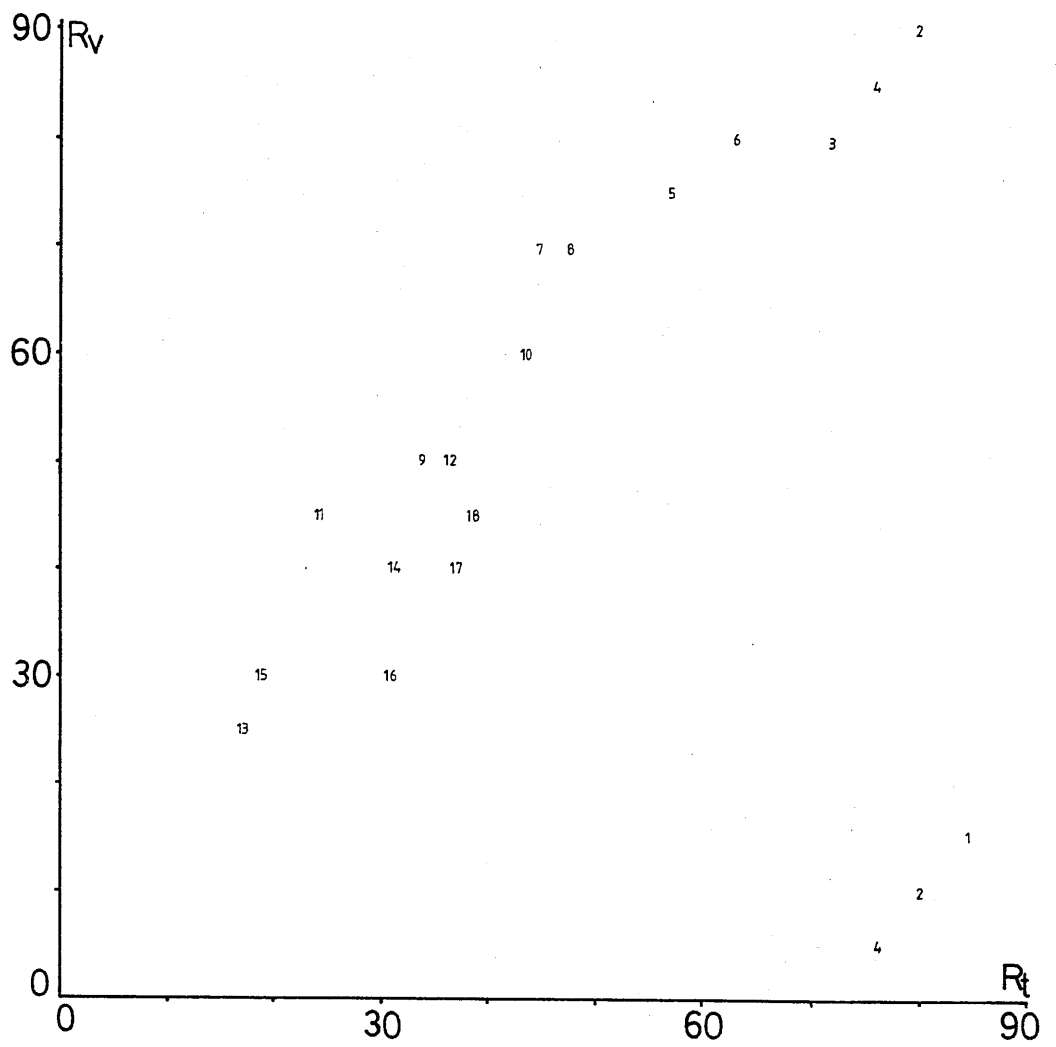
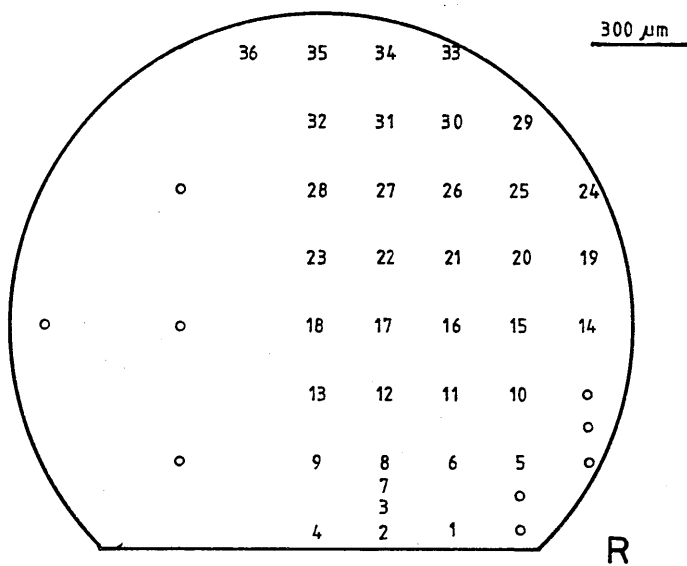
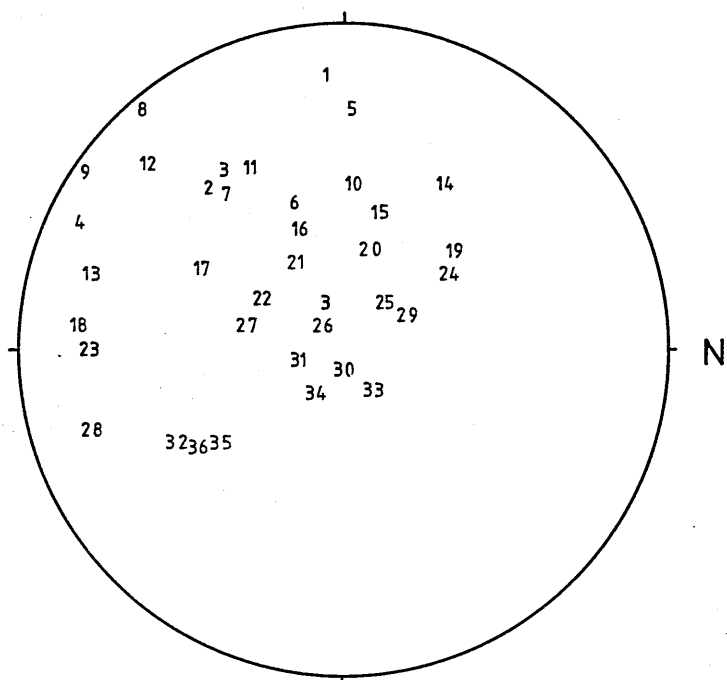


FIGURE 75c



A 33D 61

FIGURE 76a

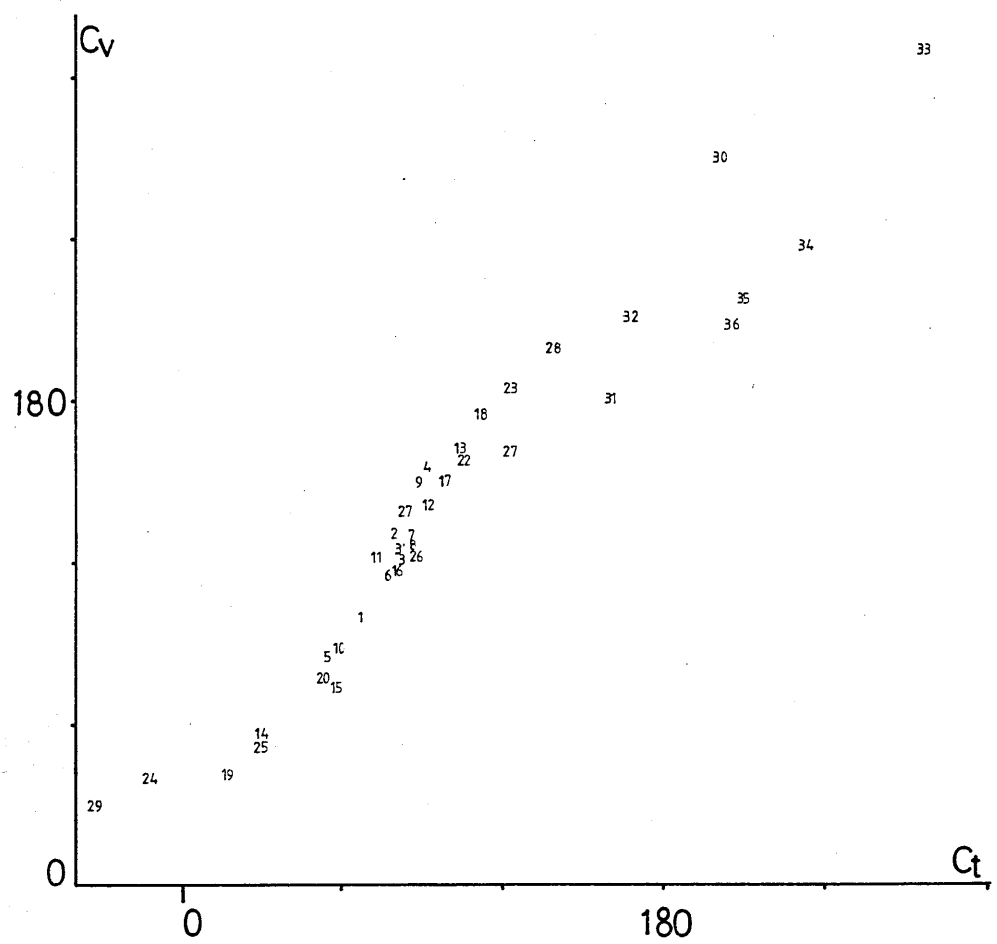


FIGURE 76b

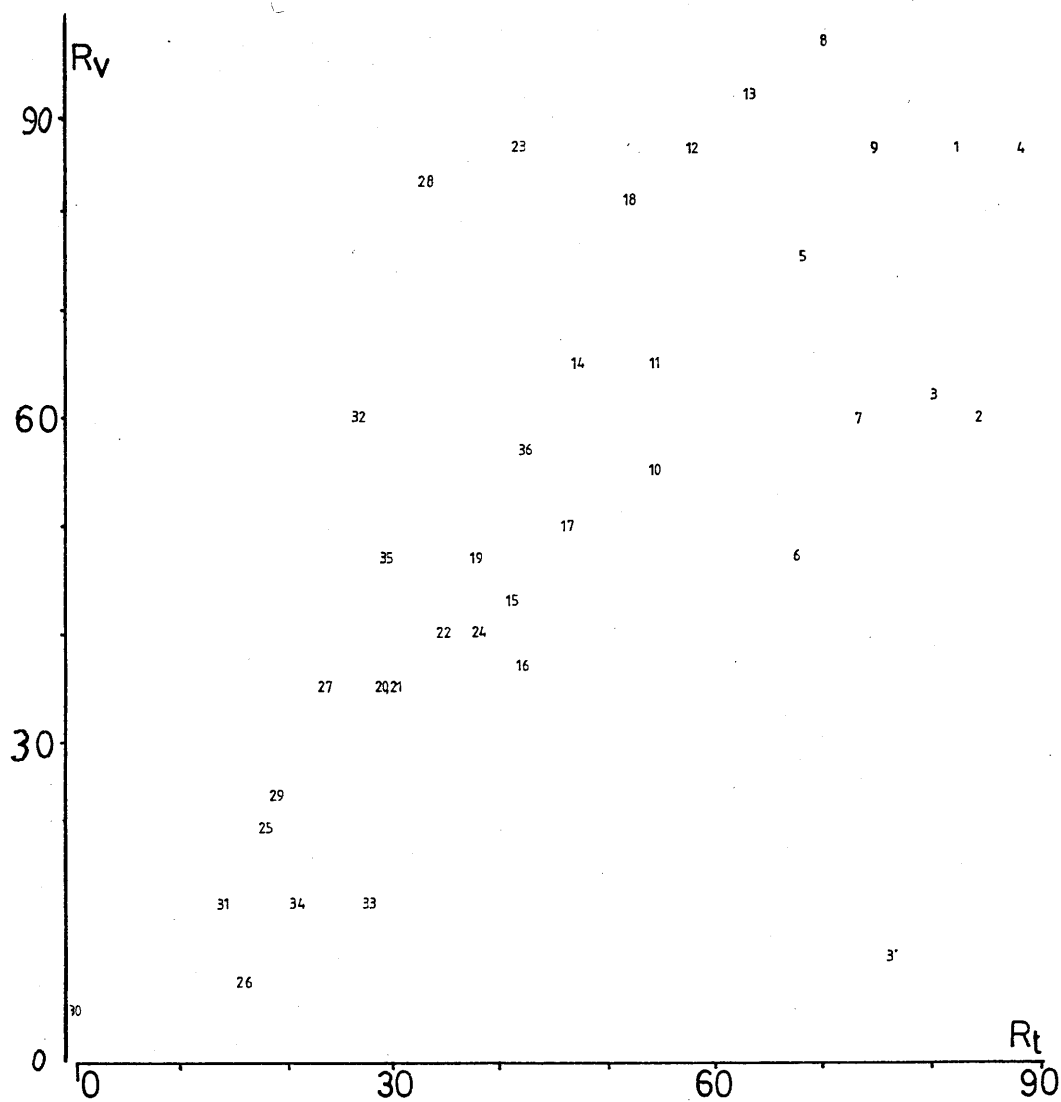
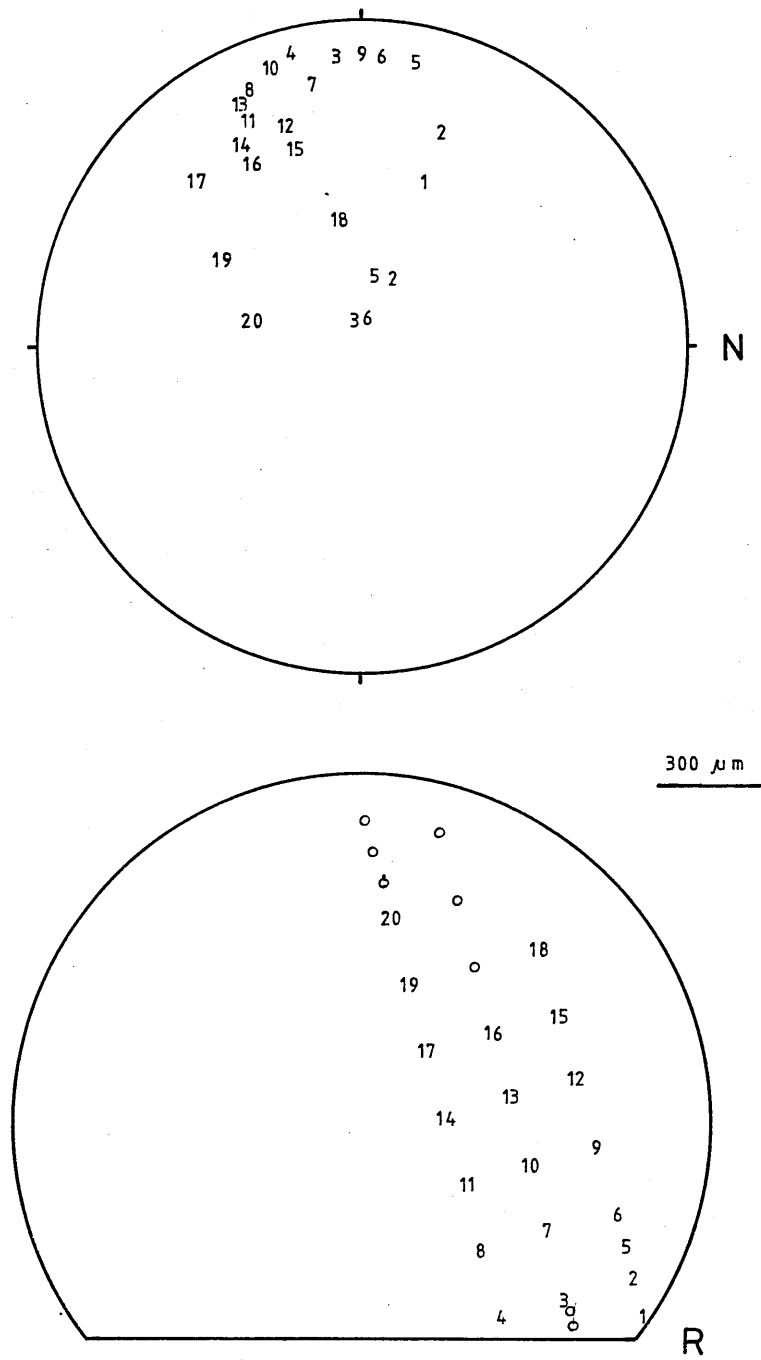


FIGURE 76c





A 33 D 131

FIGURE 77a

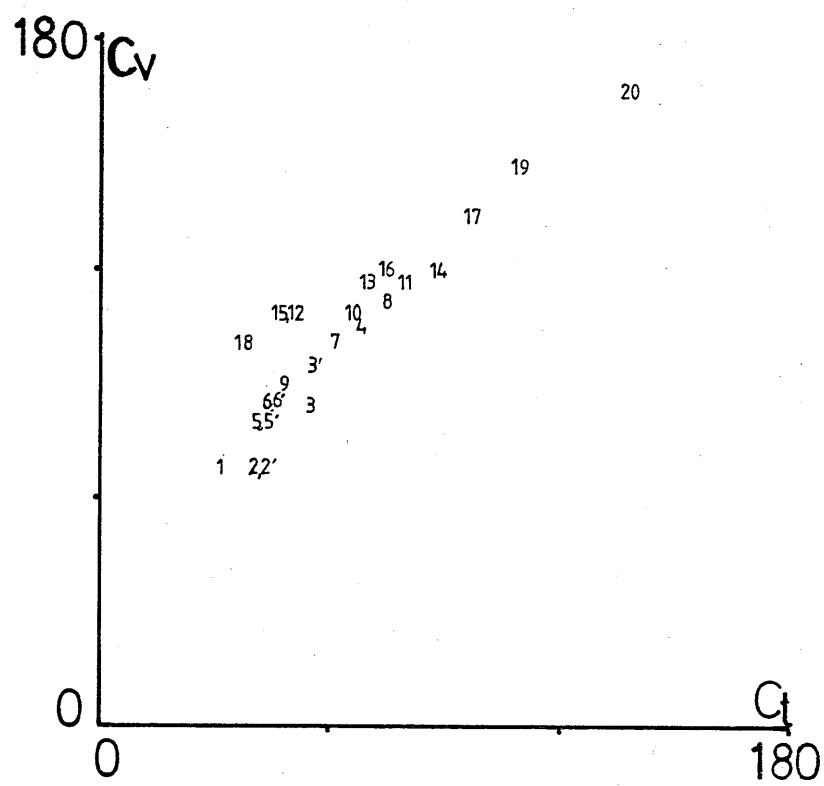


FIGURE 77b

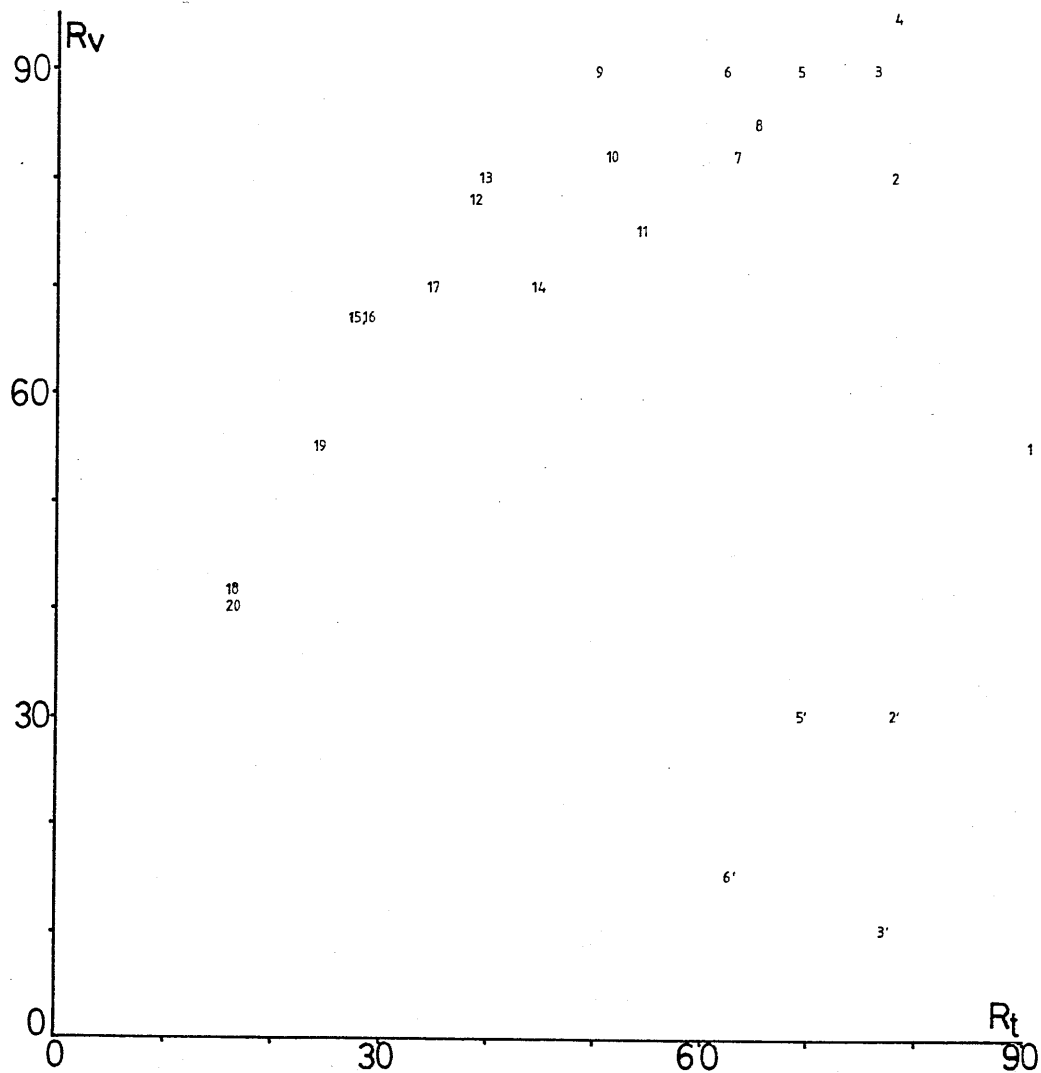
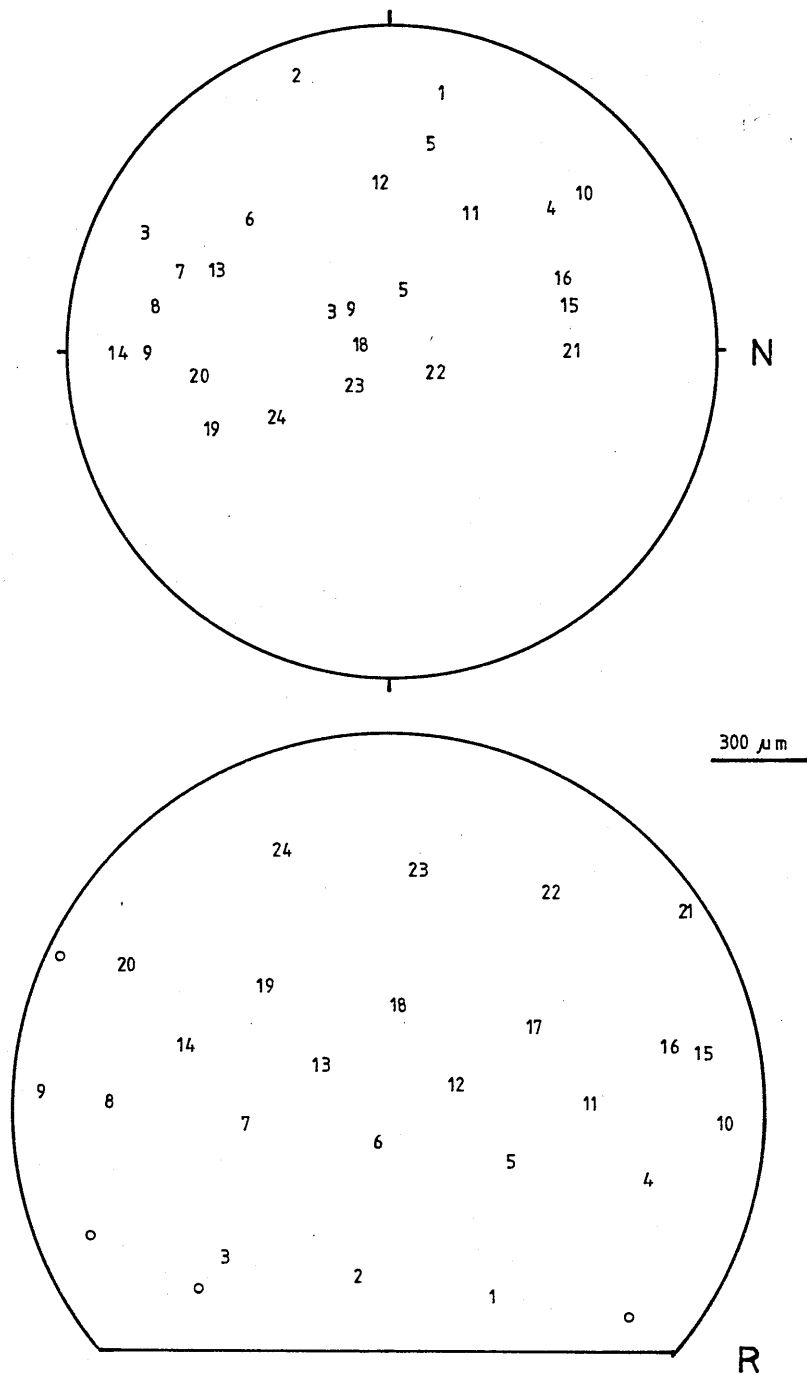


FIGURE 77c



A 33 D 146

FIGURE 78a

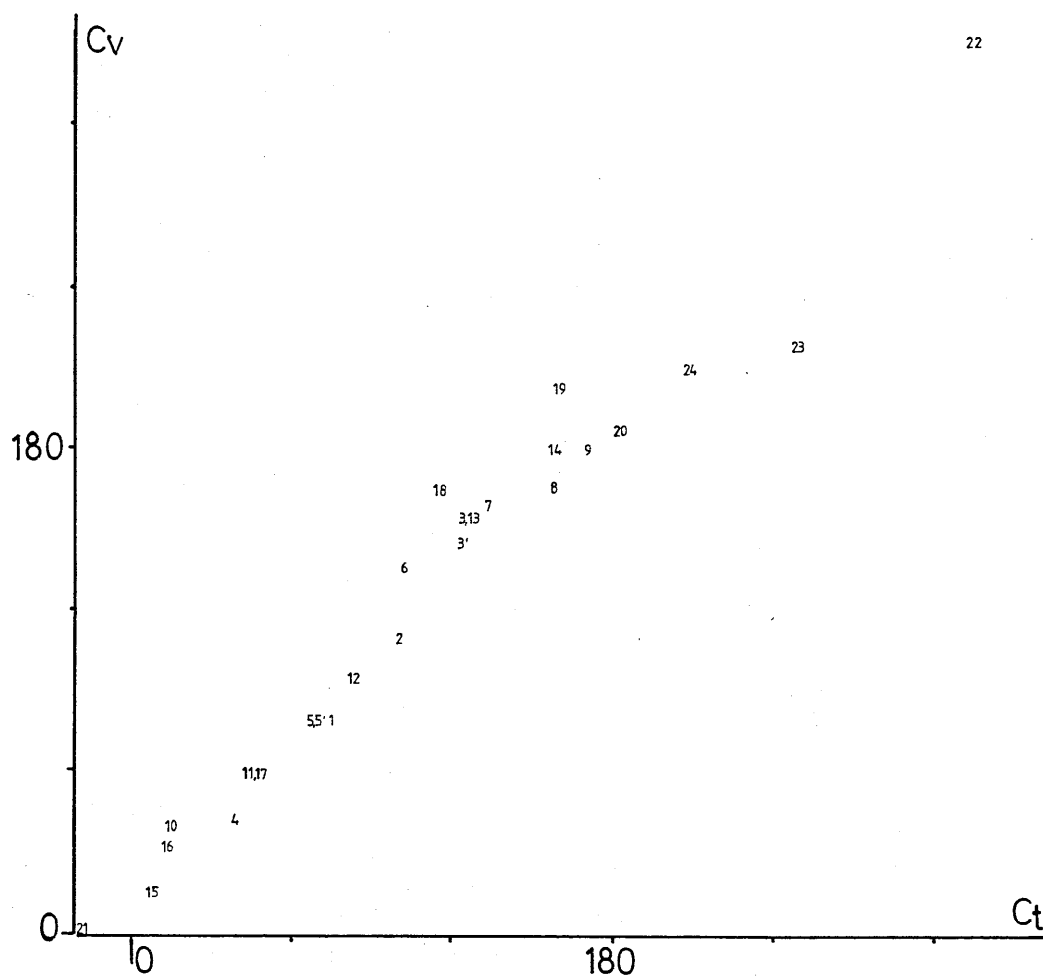


FIGURE 78b

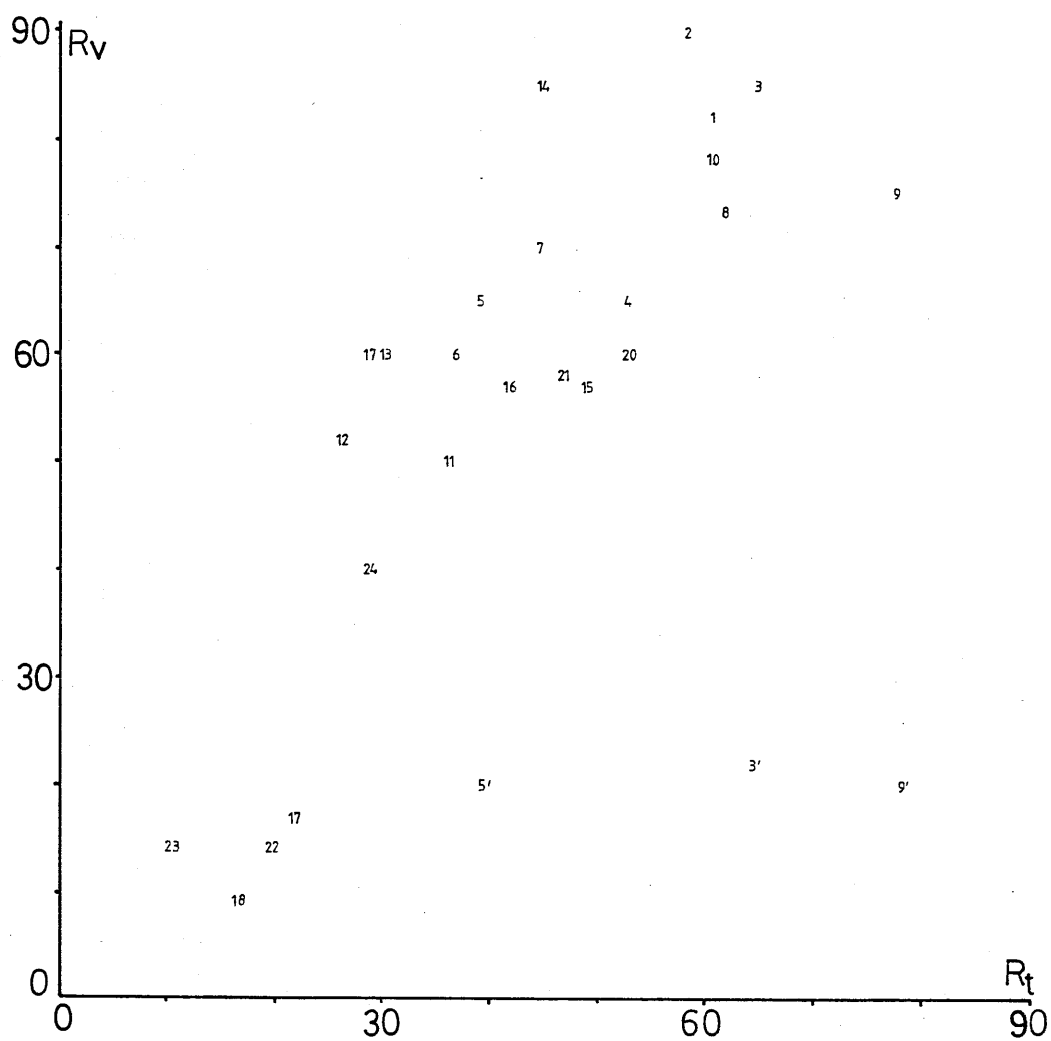


FIGURE 78c

#### FIGURES 79 - 91

The first figure in each set is a drawing of a flat - mounted retina. The boundary is the ora serrata, with incomplete radial incisions from the margin to allow flattening.

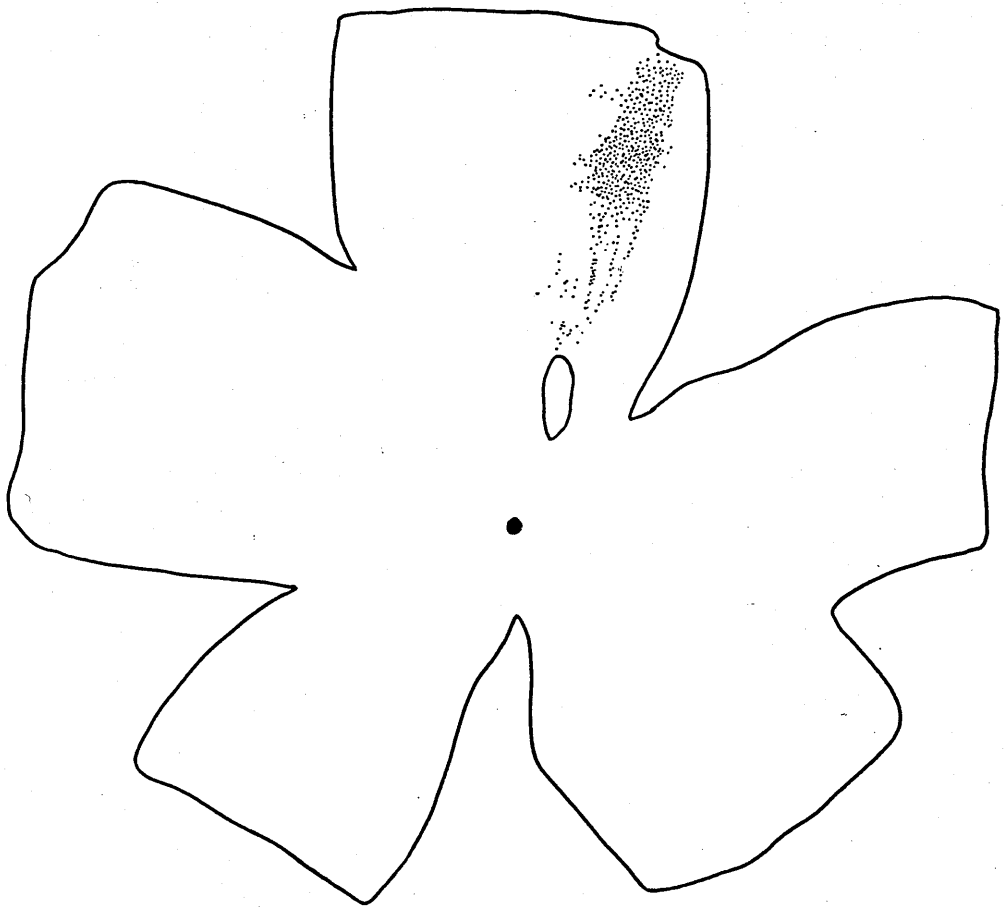
The central filled circle shows the position of the optic nerve head.

The eccentric open ring shows the lesion produced by the method explained on page 40.

Dots indicate the positions of HRP - filled ganglion cells.

Radial lines indicate the positions of HRP - filled axons in those specimens in which cell bodies were not visibly stained.

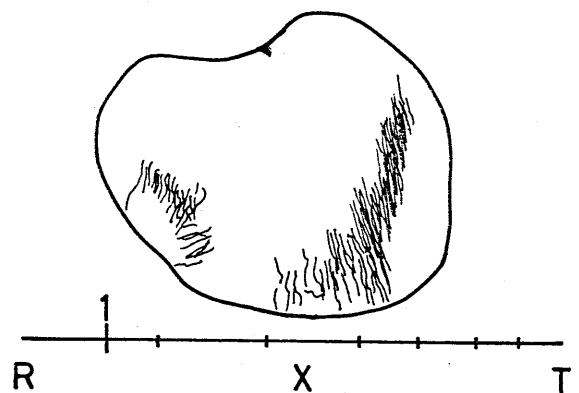
In each set the succeeding drawings show orthogonal cross - sections of the optic pathway. Page 148 explains the conventions used.



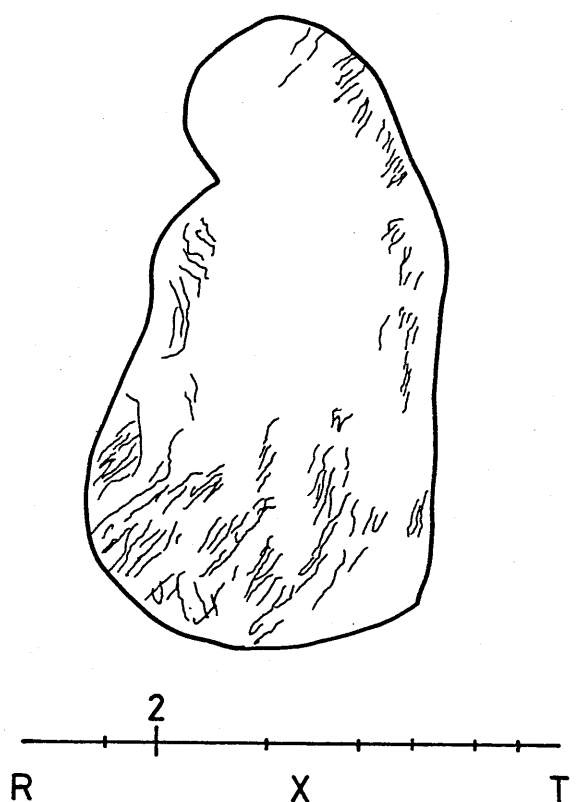
DORSAL SECTOR

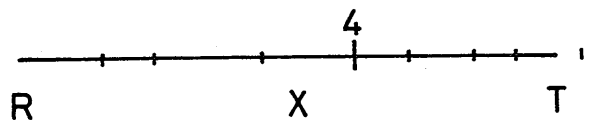
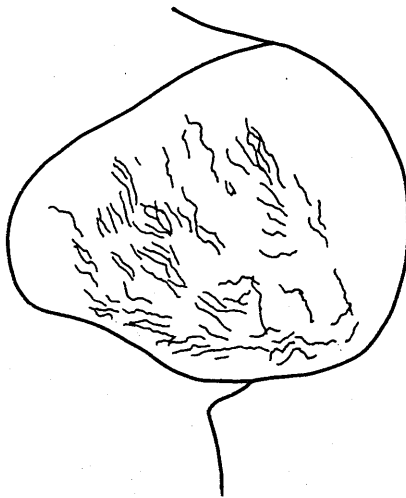
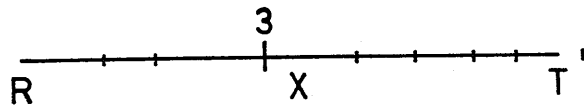
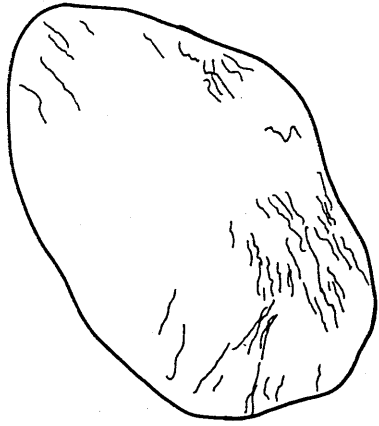
FIGURE 79

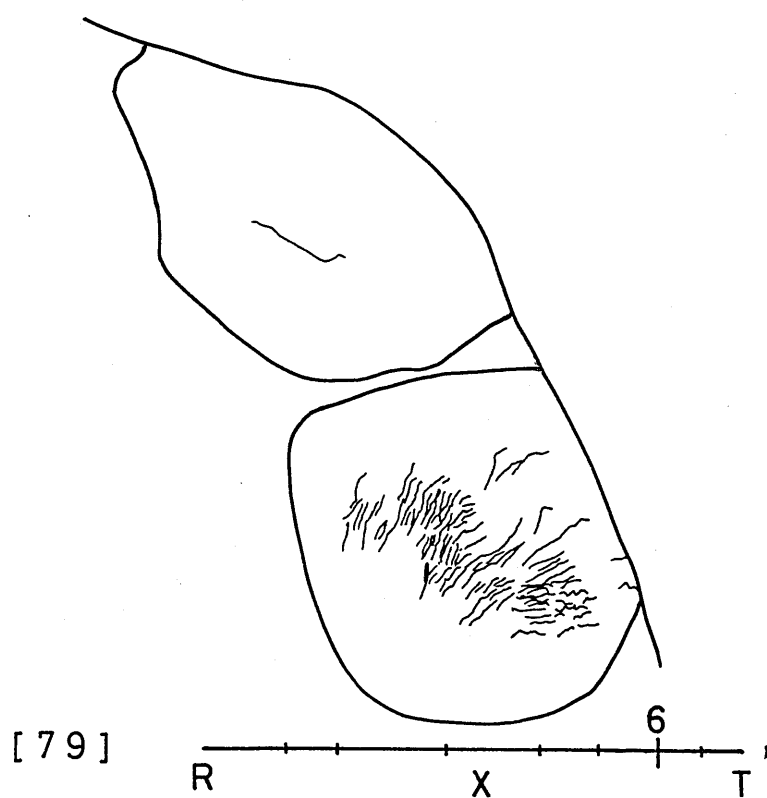
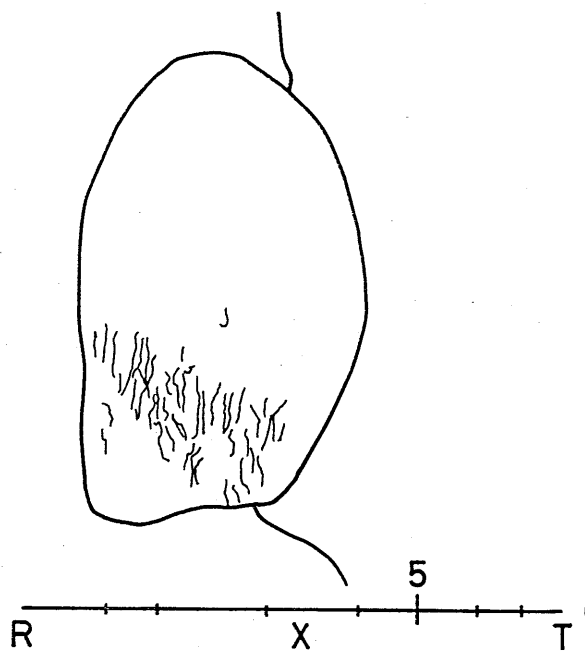


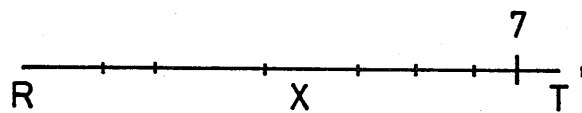
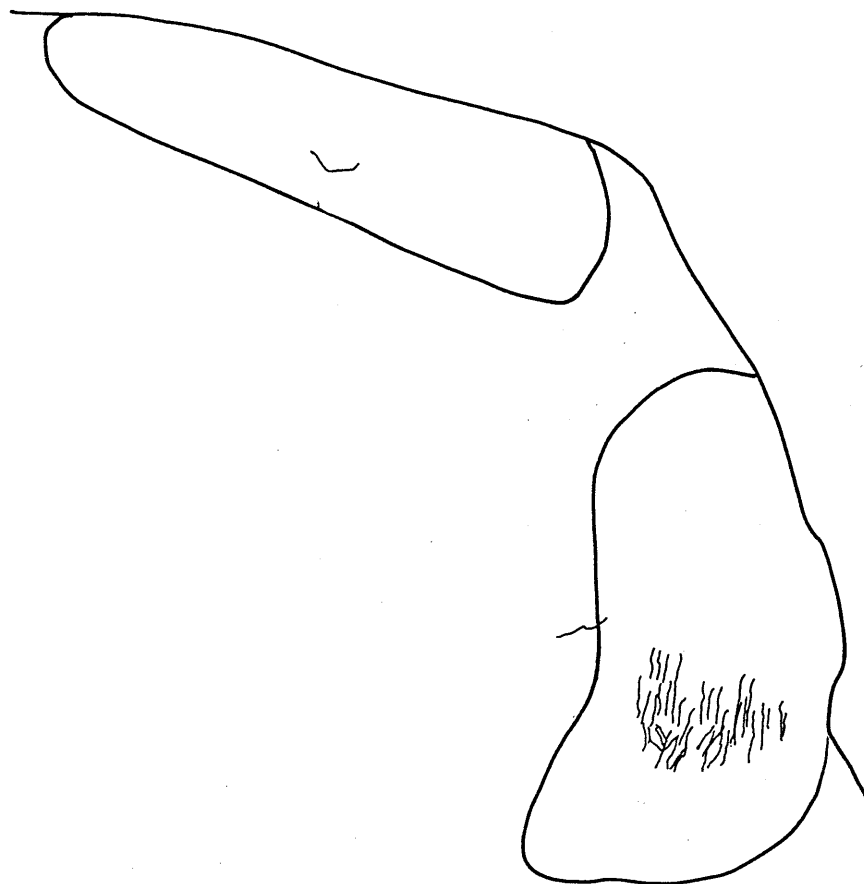


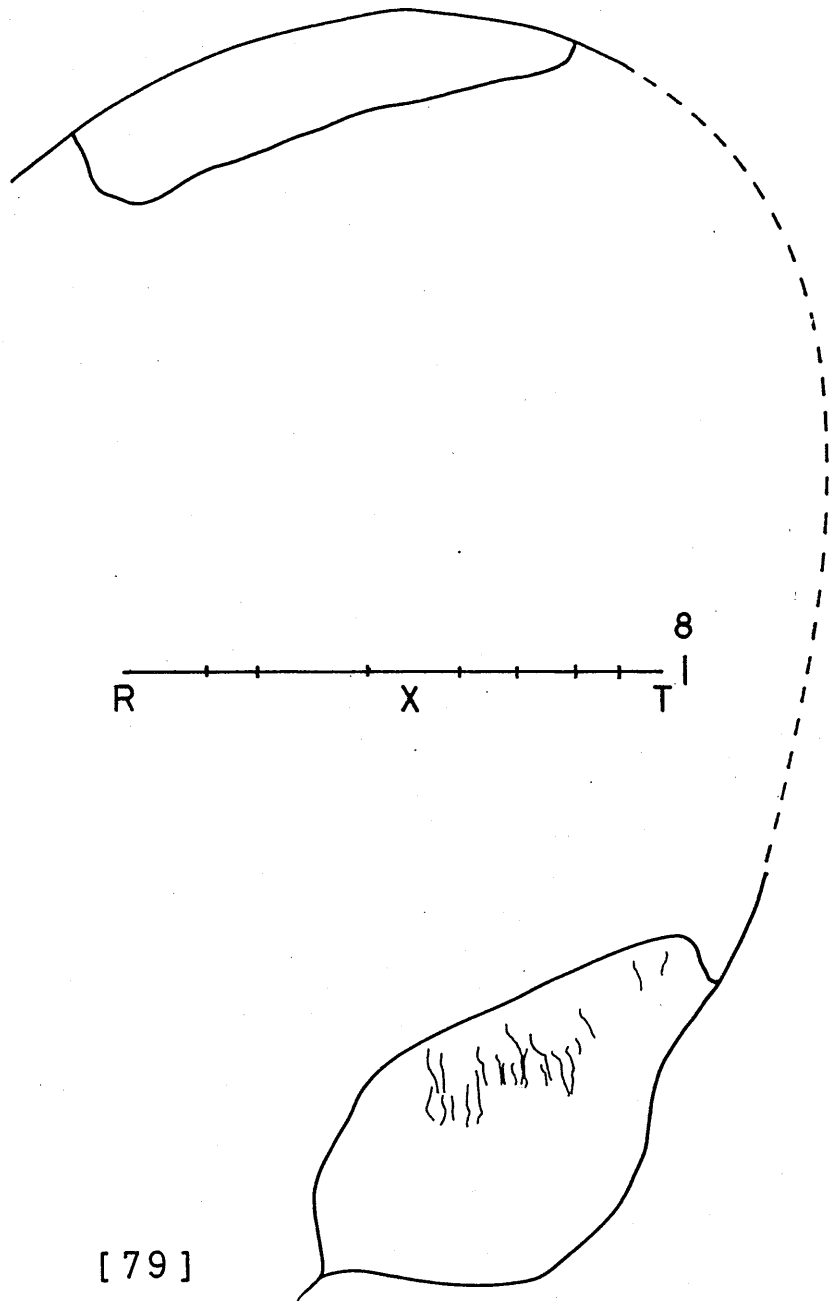
NORMAL OPTIC PATHWAY : DORSAL SECTOR 1



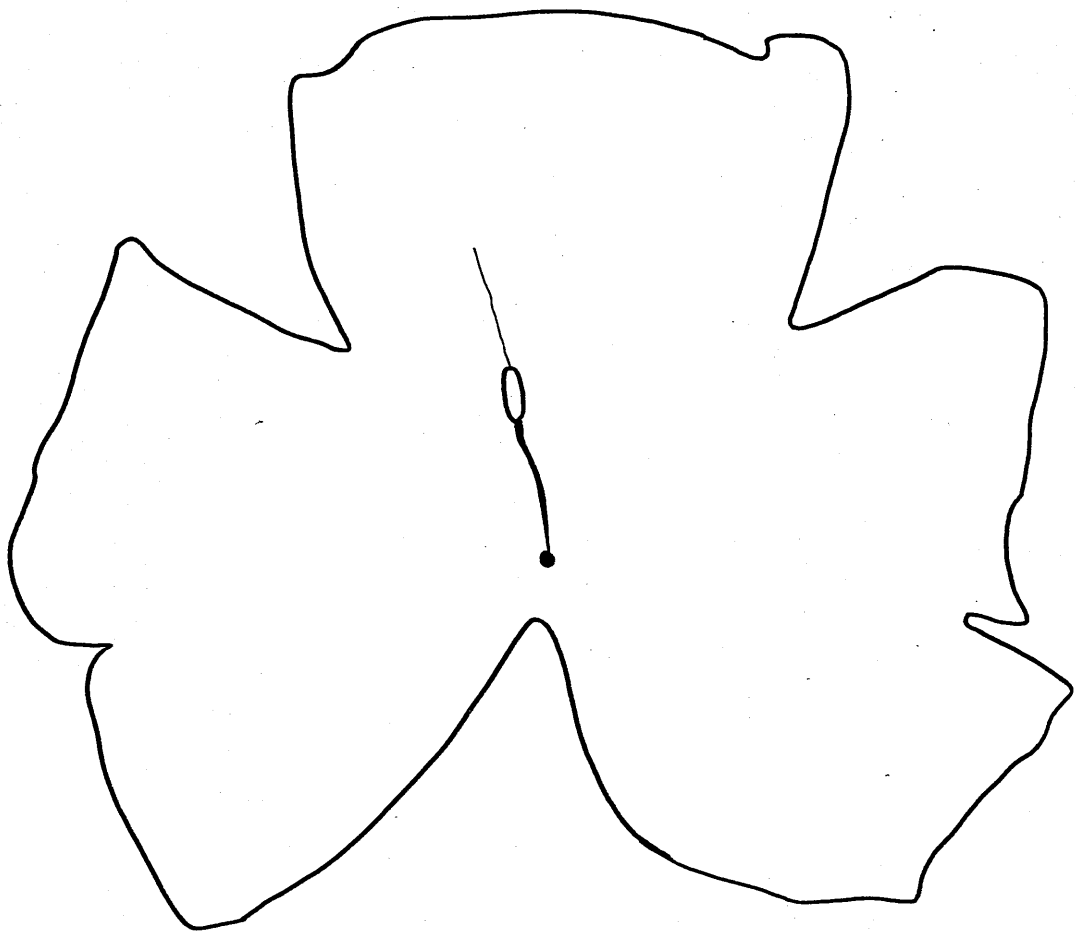








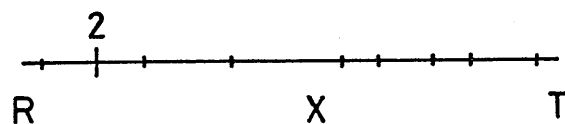
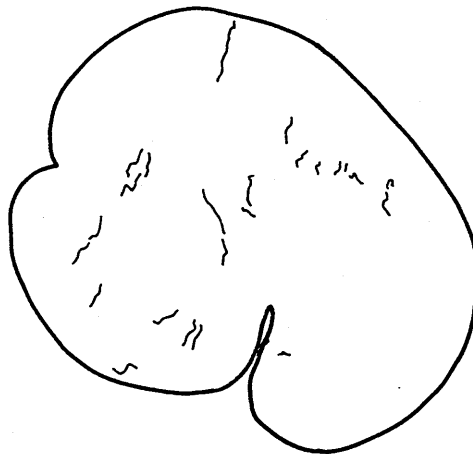
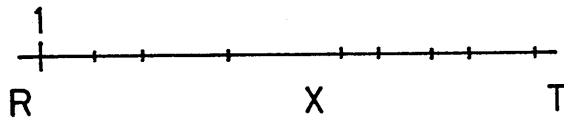
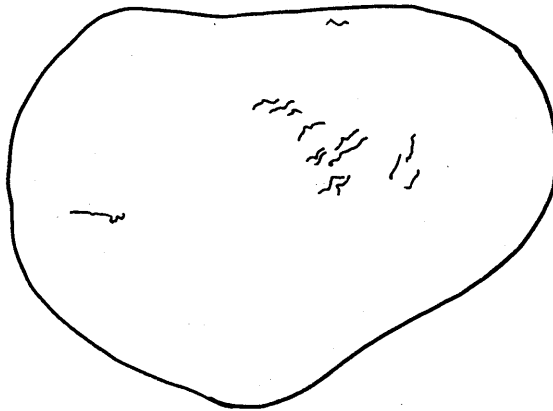
[ 79 ]



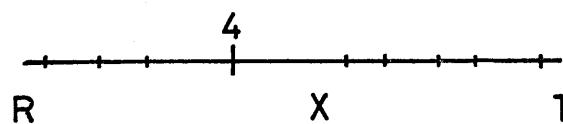
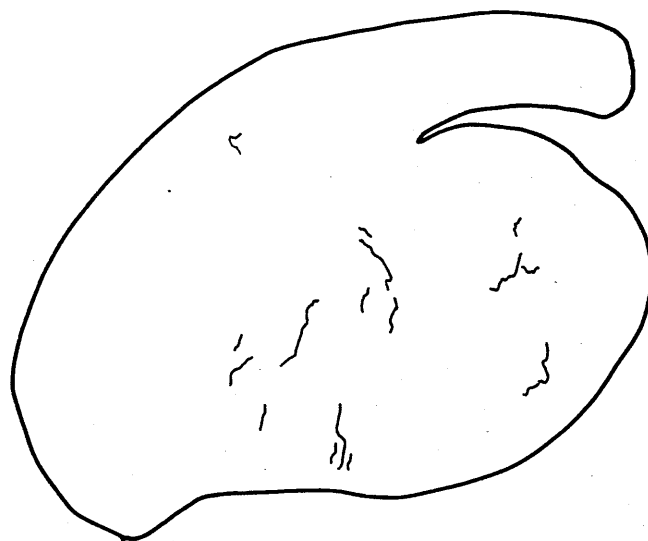
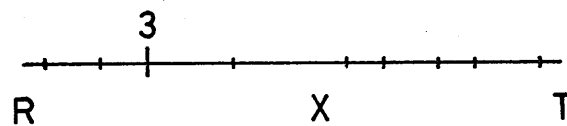
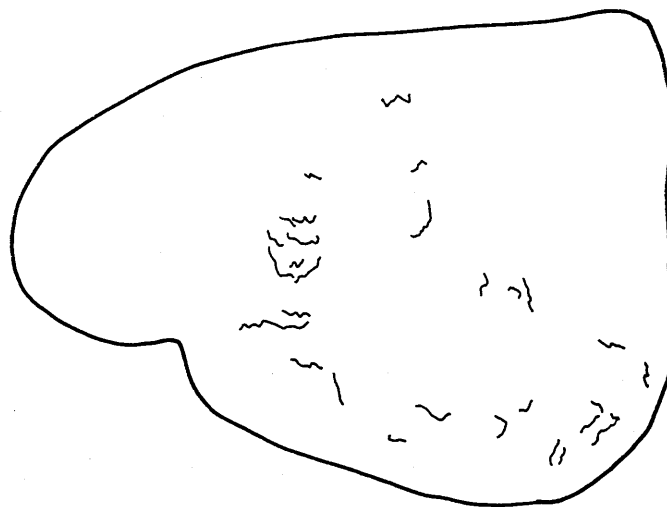
DORSAL SECTOR

FIGURE 80

# NORMAL OPTIC PATHWAY : DORSAL SECTOR 2

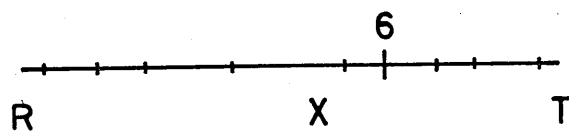
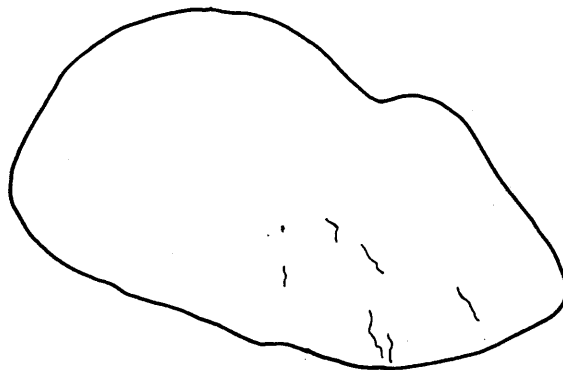
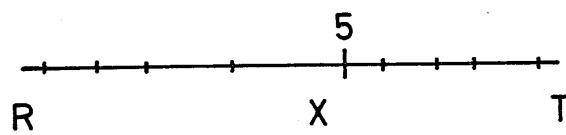
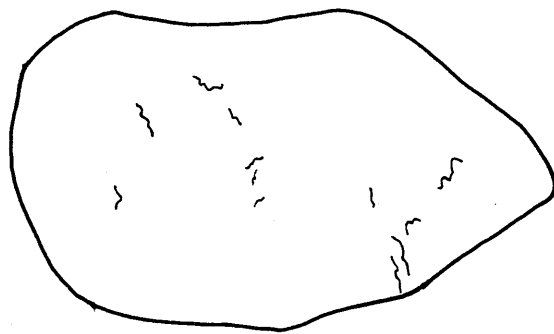


[80]

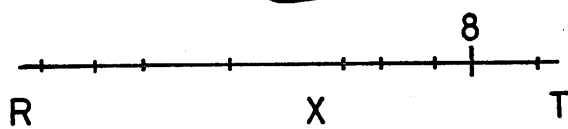
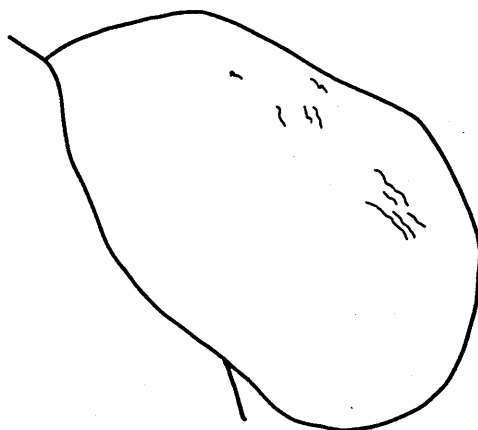
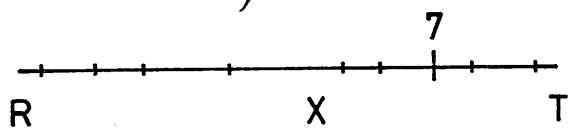
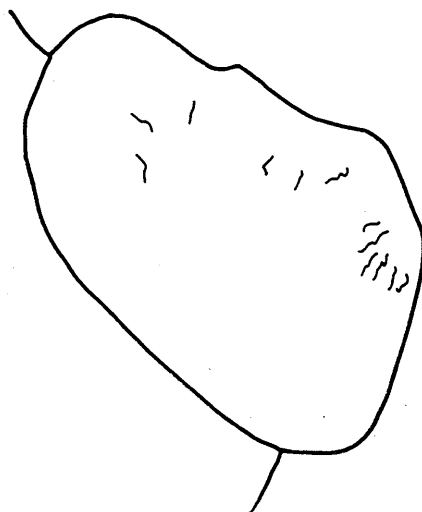


[80]

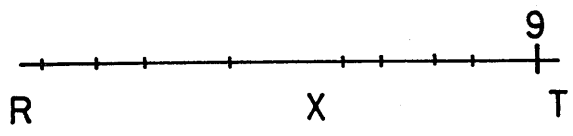
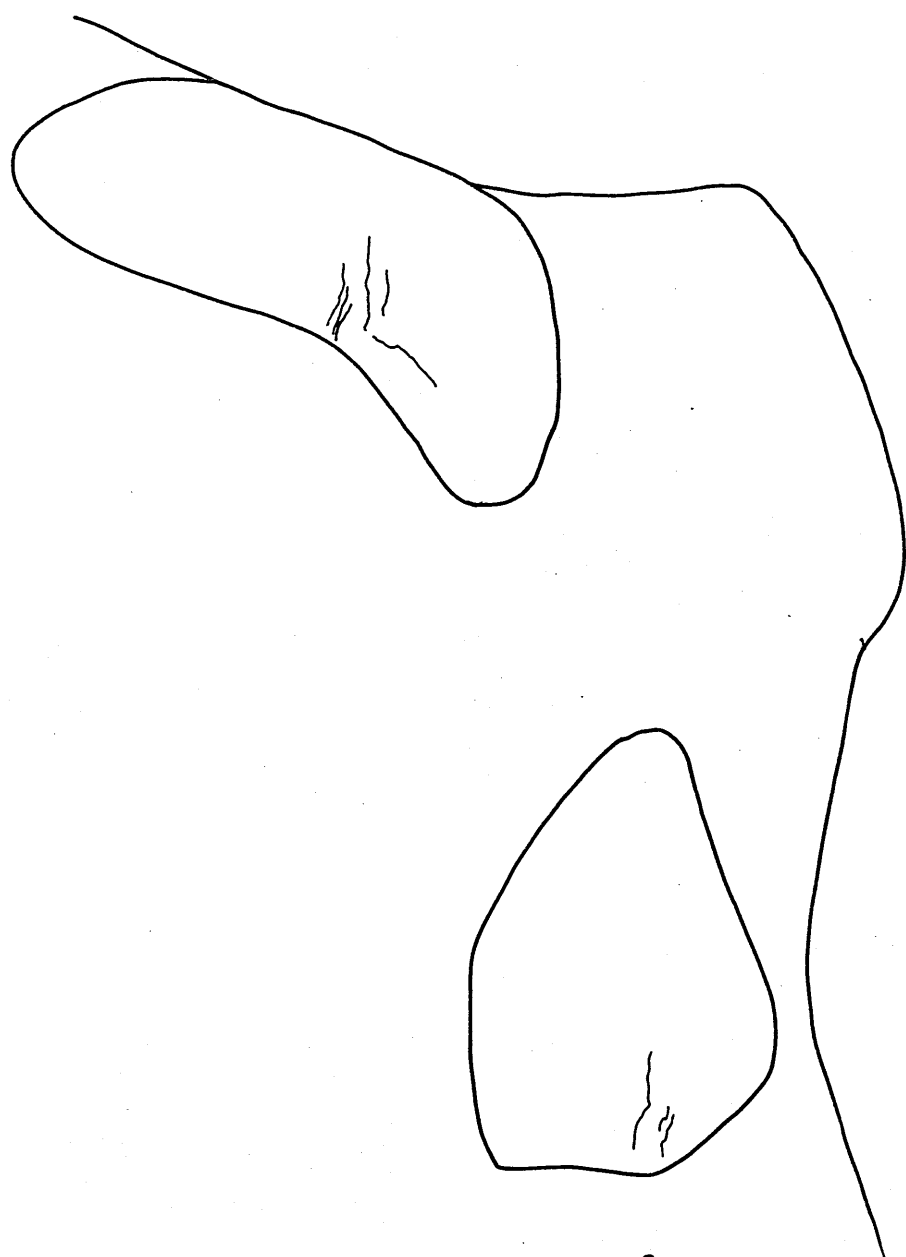




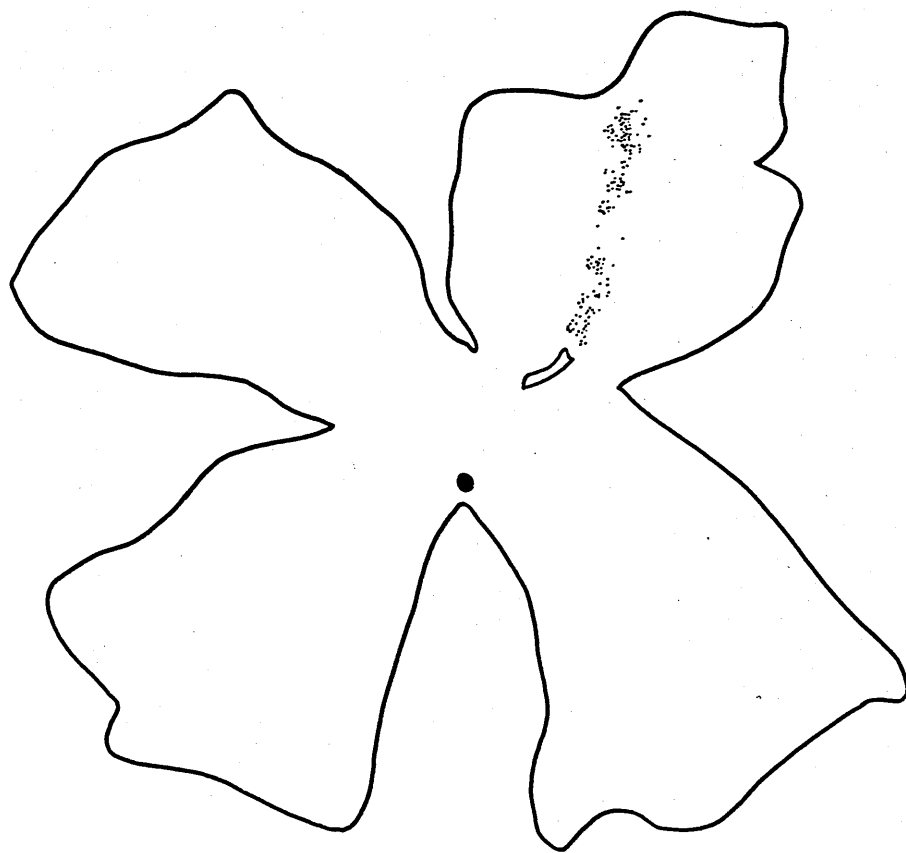
[80]



[80]



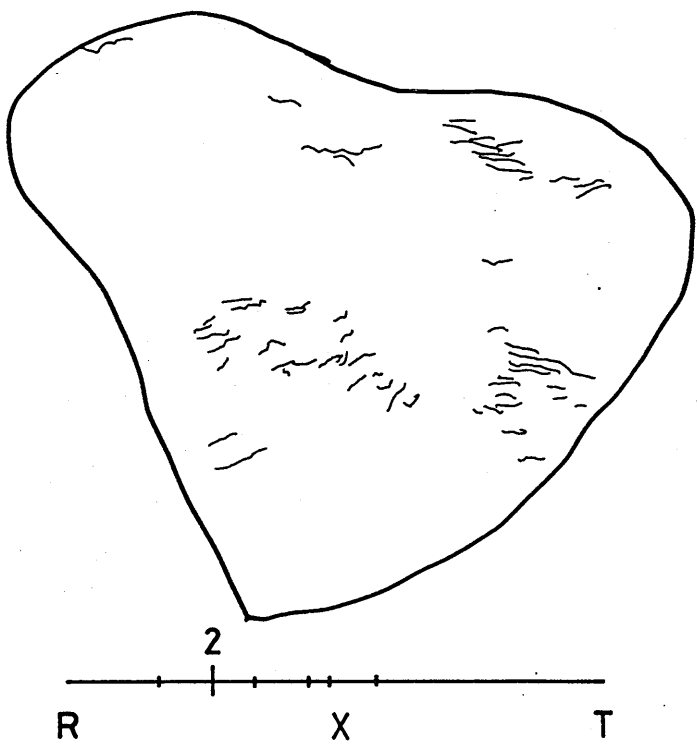
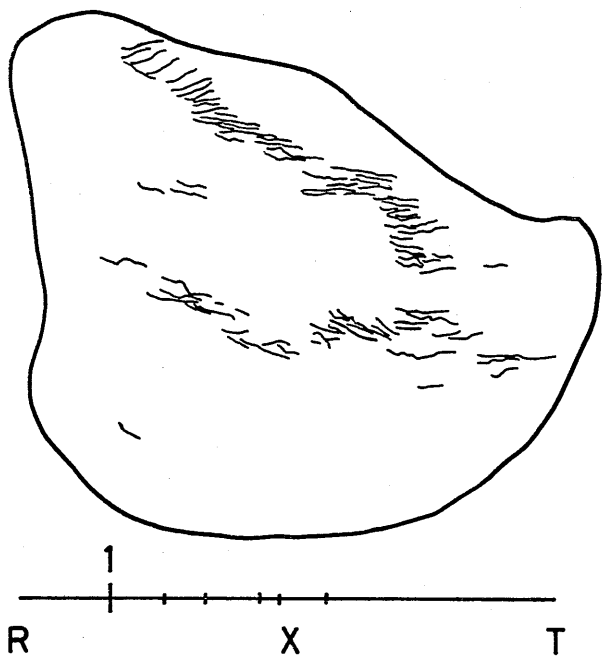
[ 80 ]

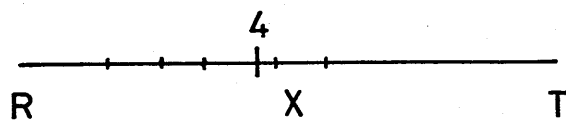
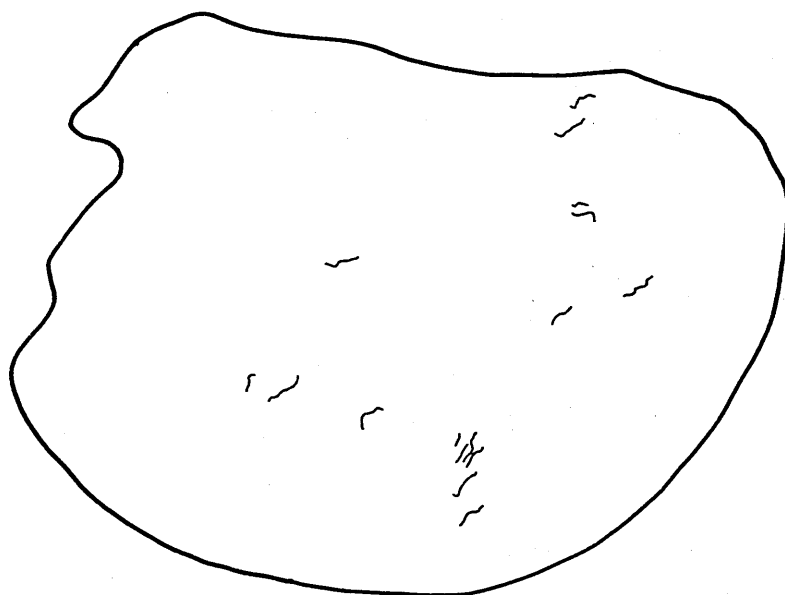
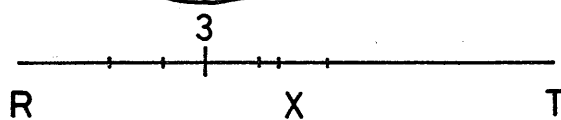
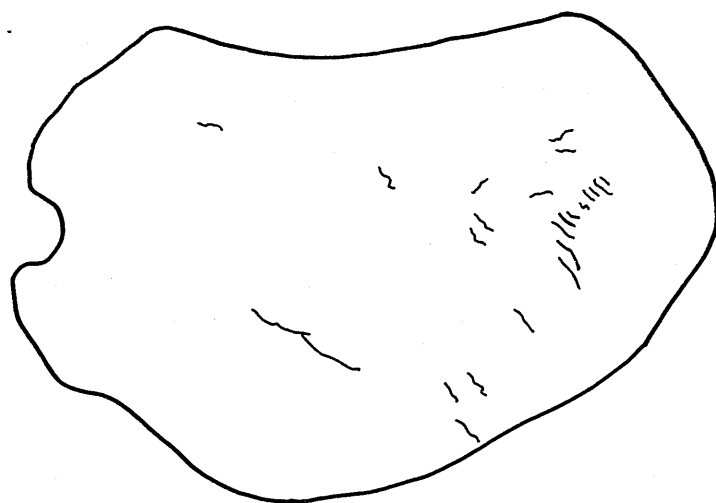


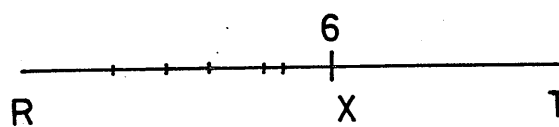
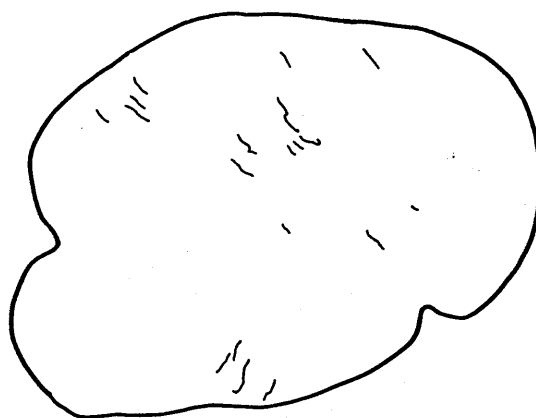
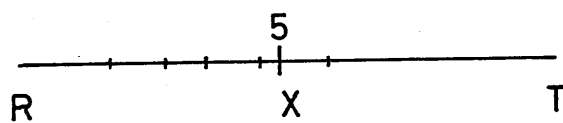
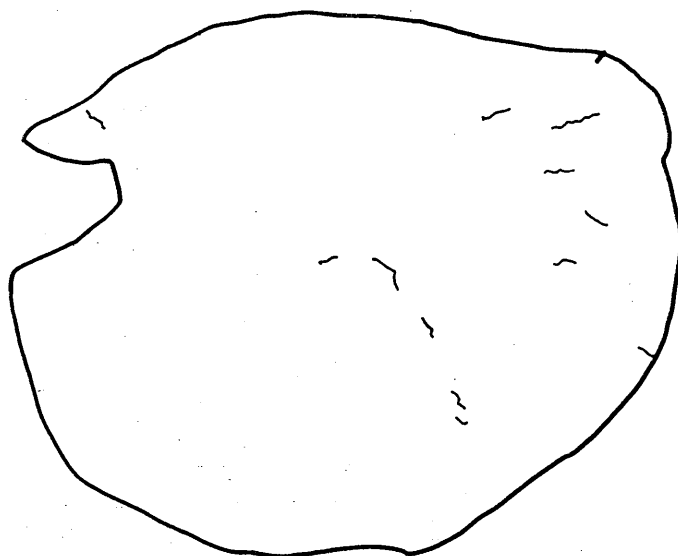
DORSO - NASAL SECTOR

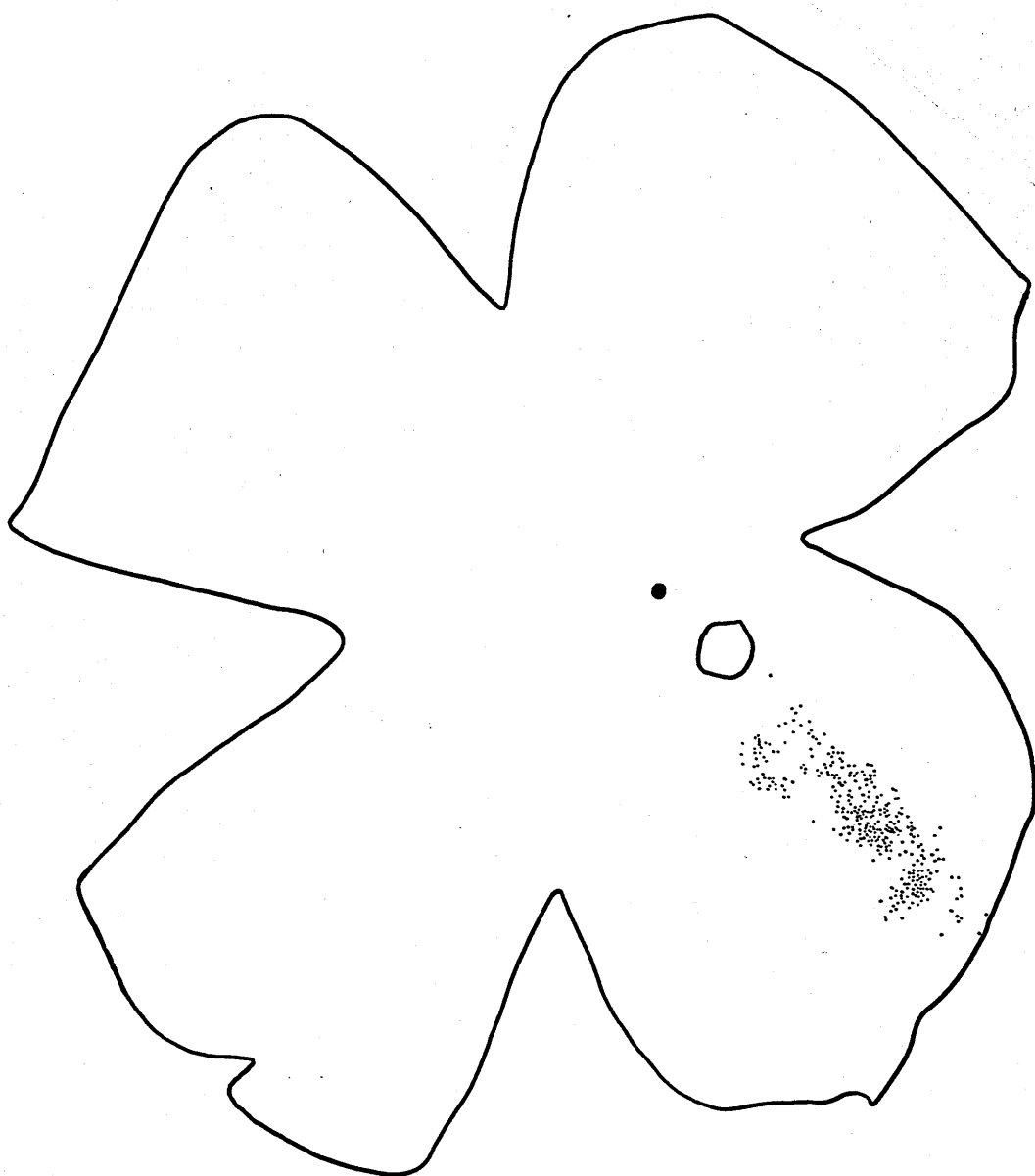
FIGURE 81

NORMAL PATHWAY : DORSONASAL SECTOR









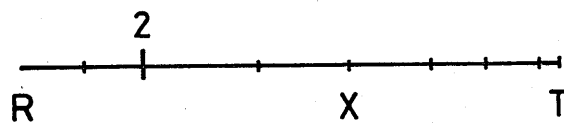
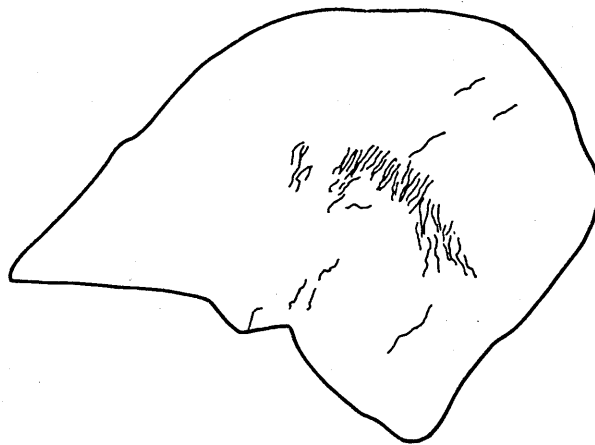
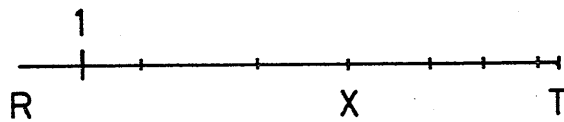
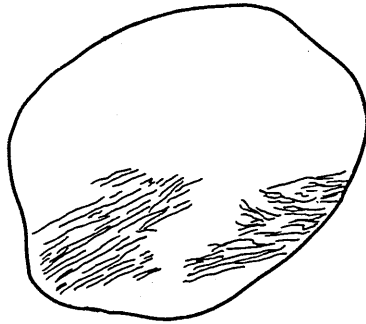
VENTRO-NASAL SECTOR

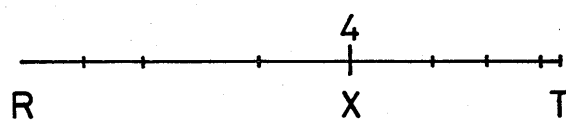
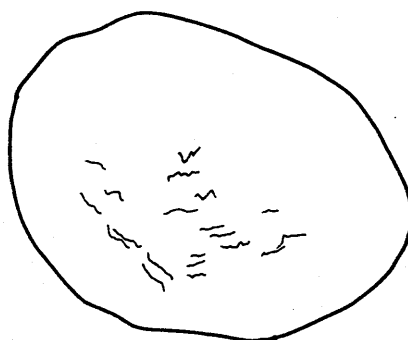
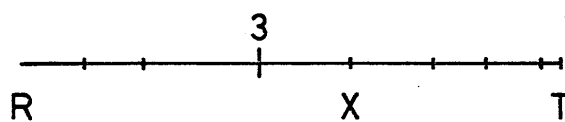
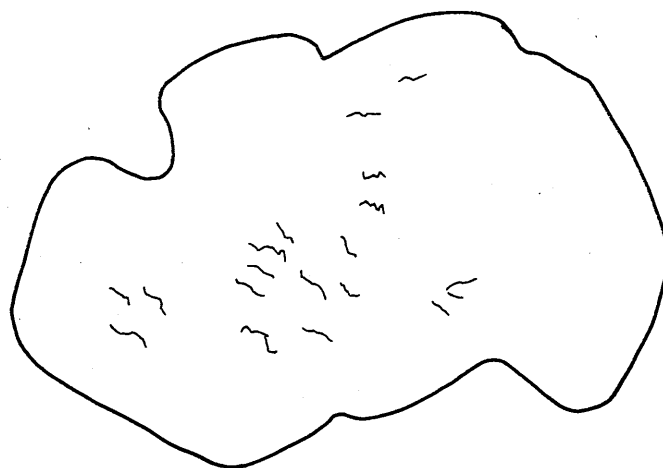
FIGURE 82

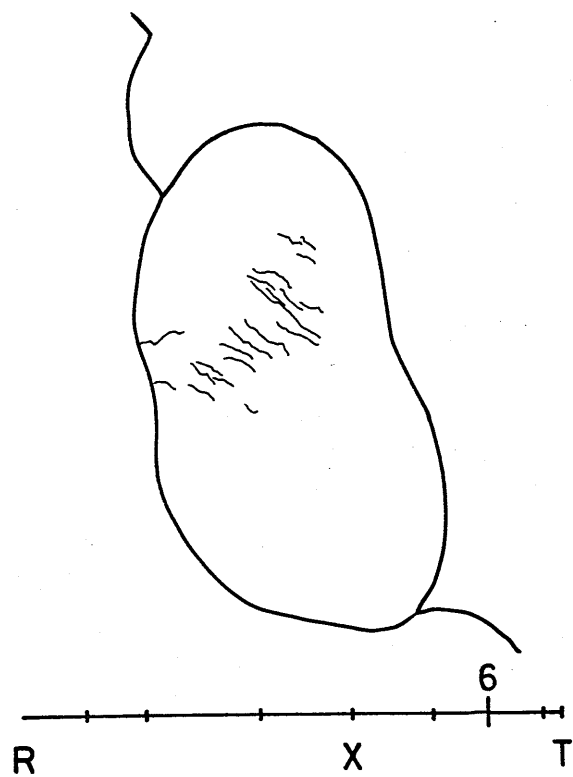
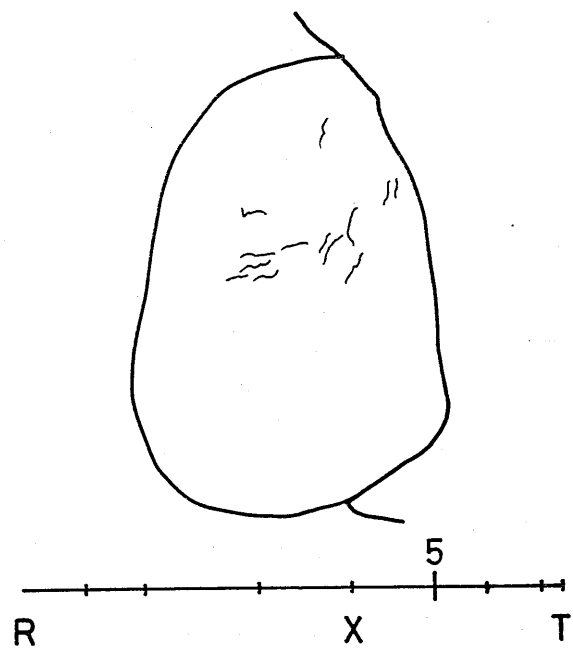


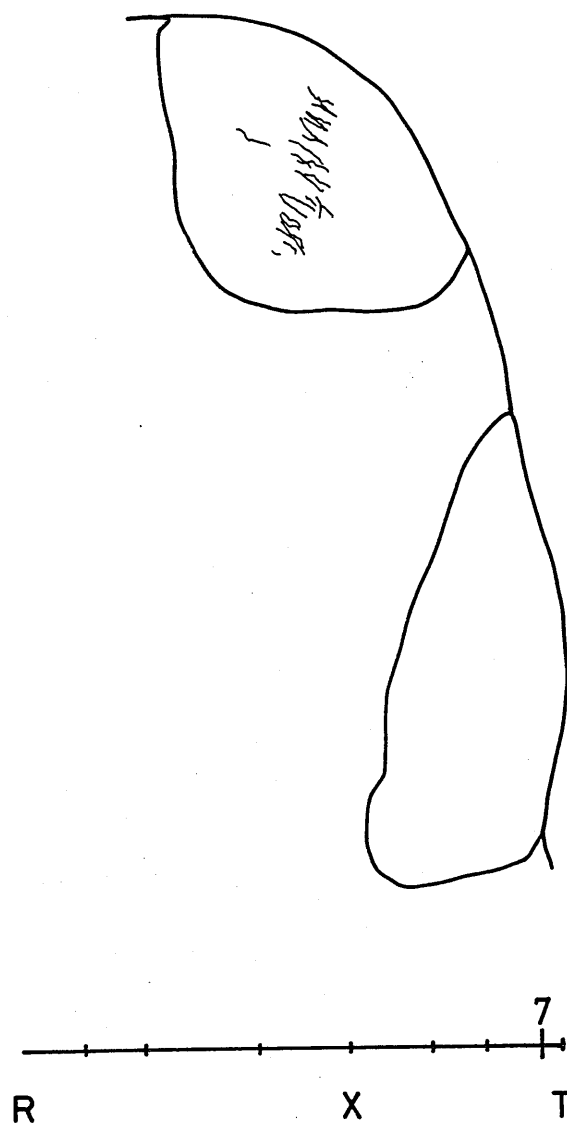
NORMAL OPTIC PATHWAY :

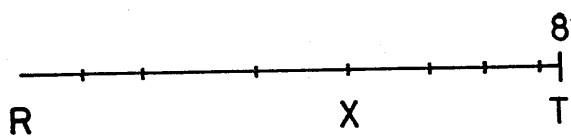
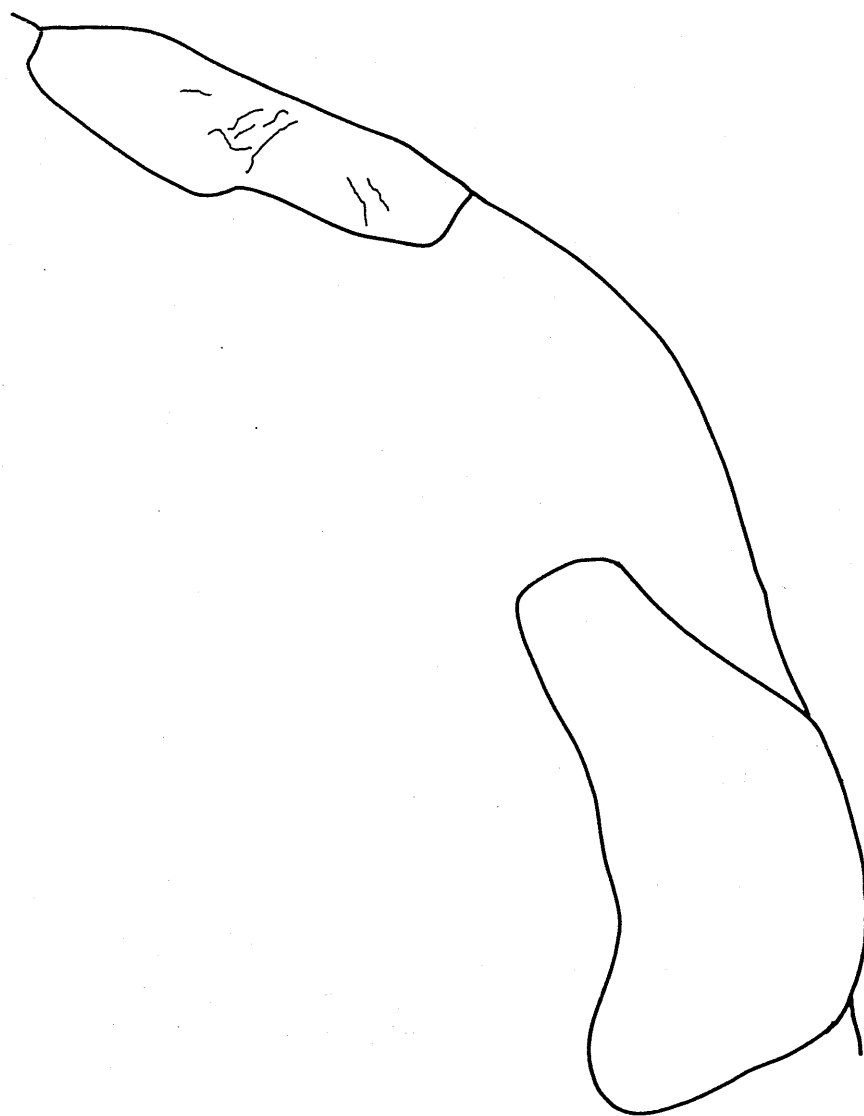
VENTRO-NASAL SECTOR 1

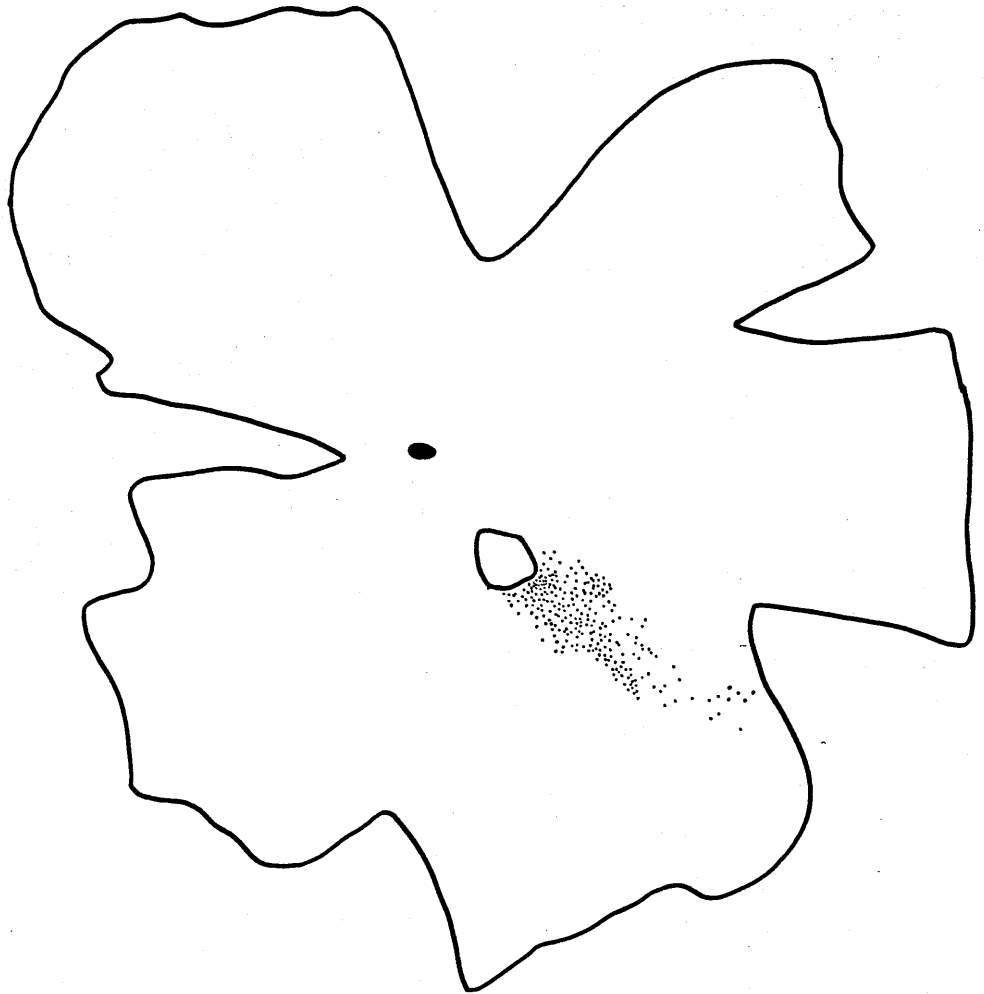










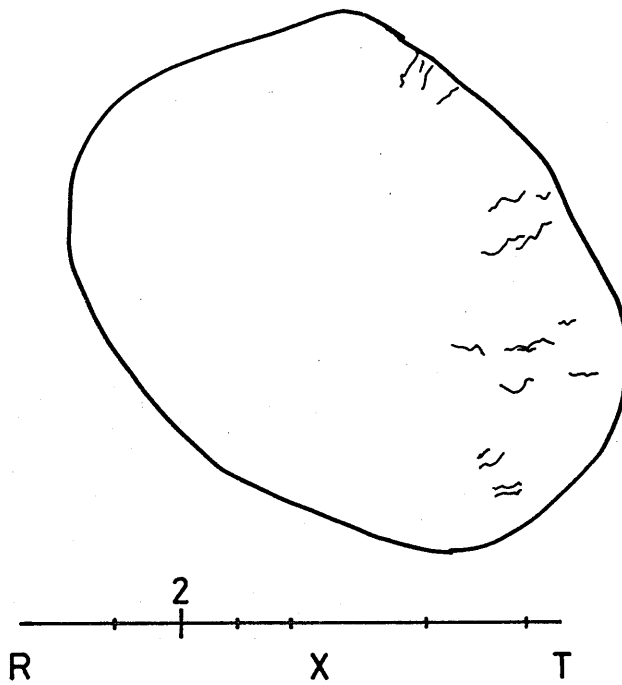
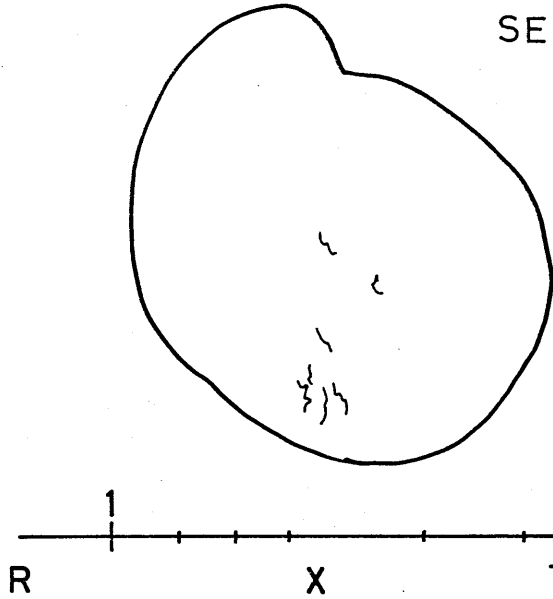


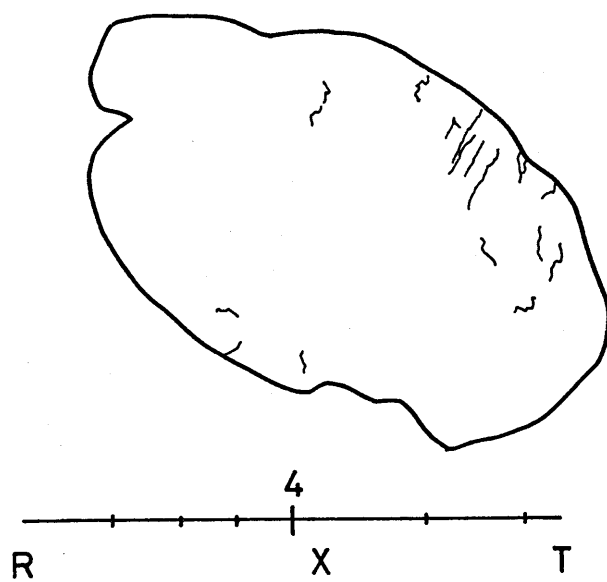
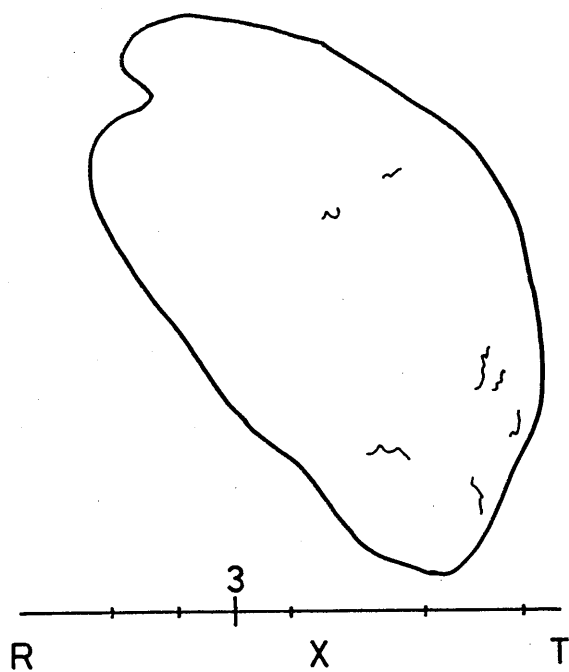
VENTRO-NASAL SECTOR

FIGURE 83

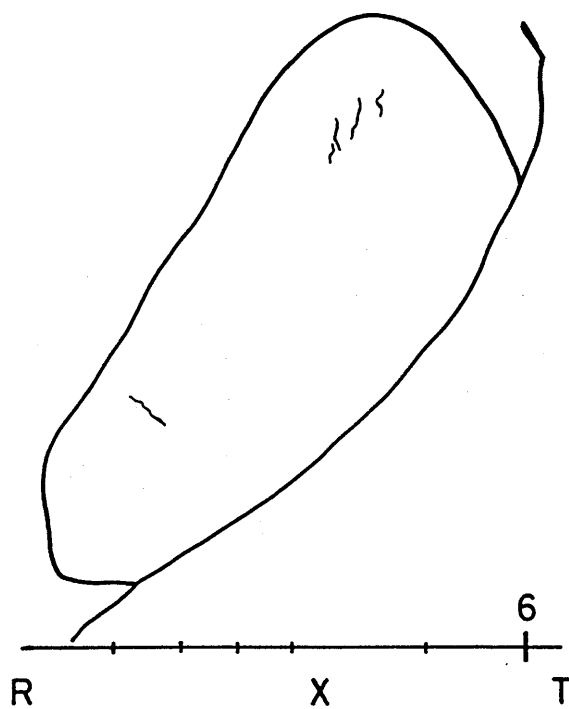
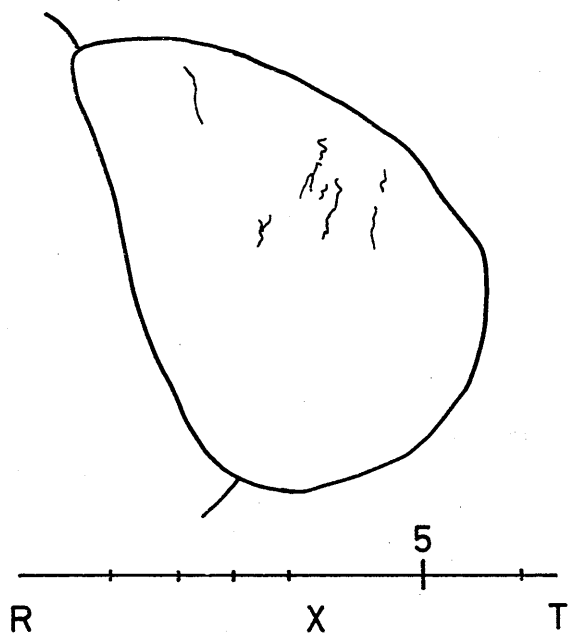
NORMAL OPTIC PATHWAY : VENTRO-NASAL

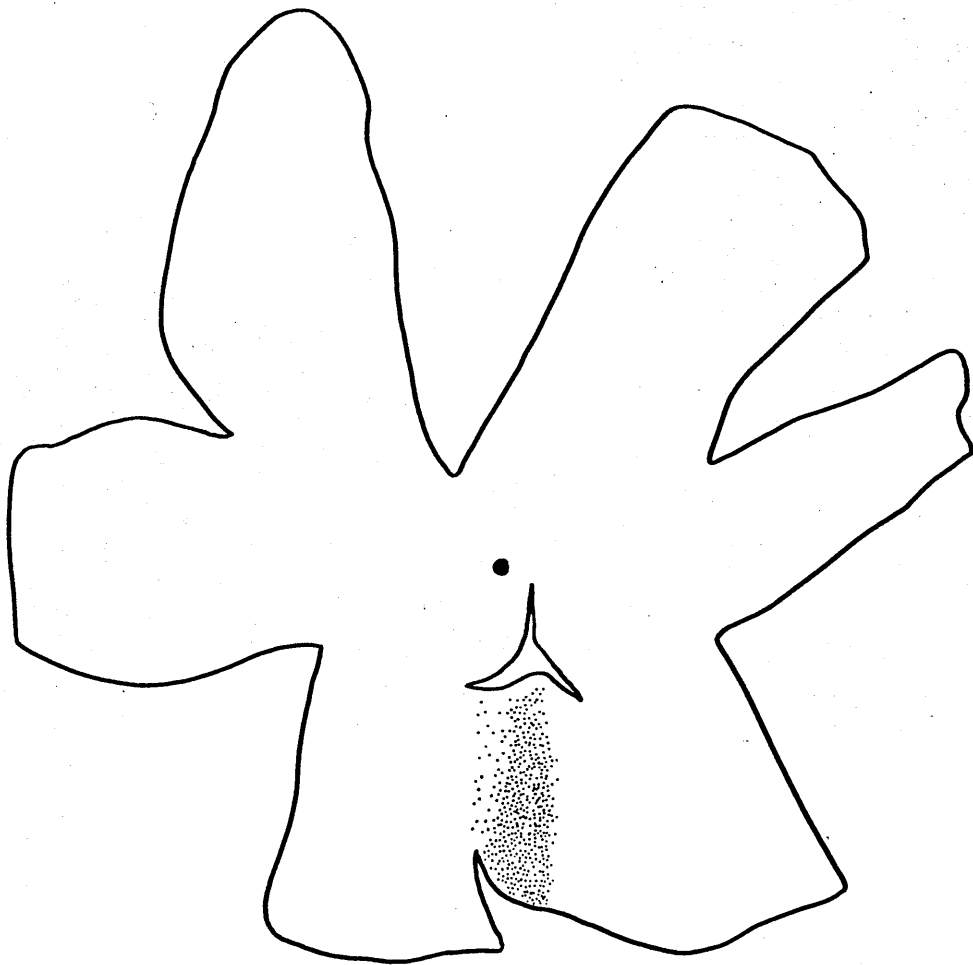
SECTOR 2





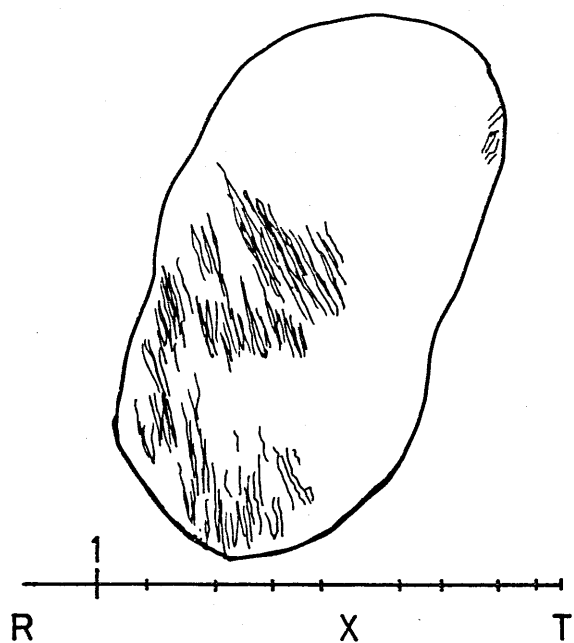




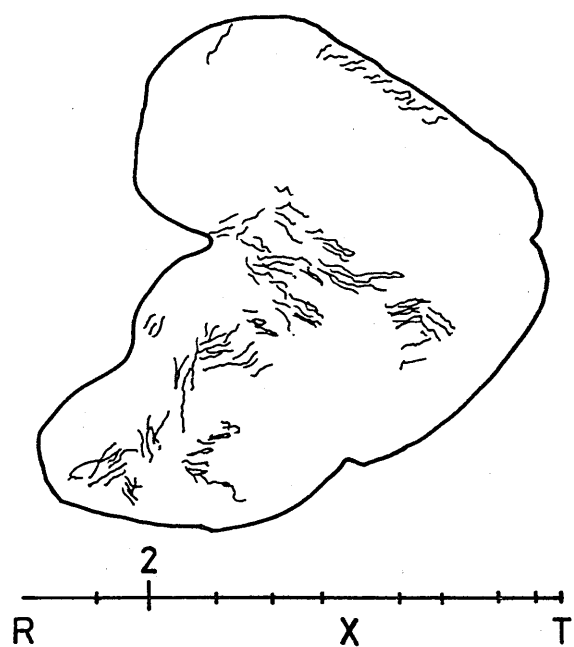


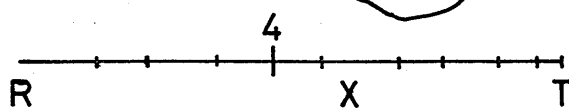
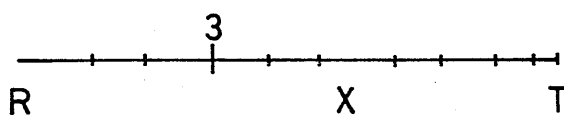
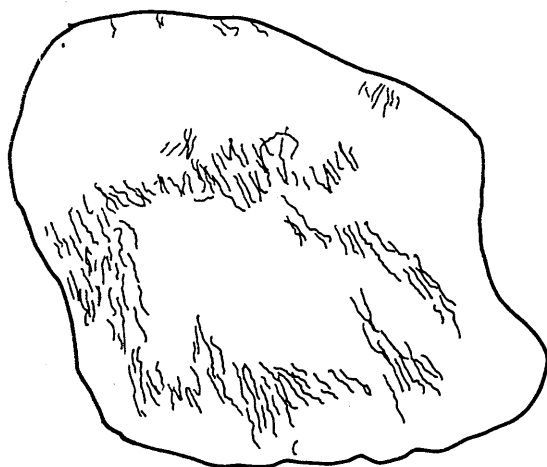
VENTRAL SECTOR

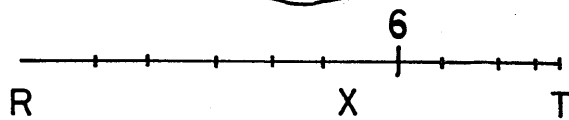
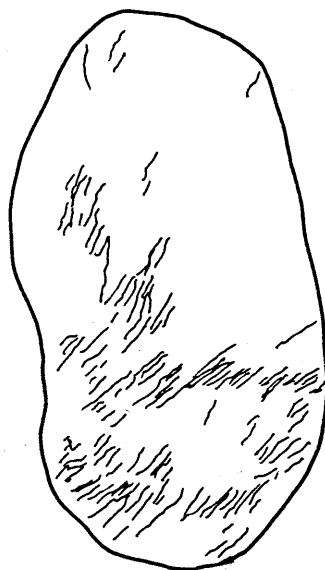
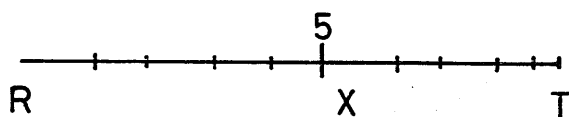
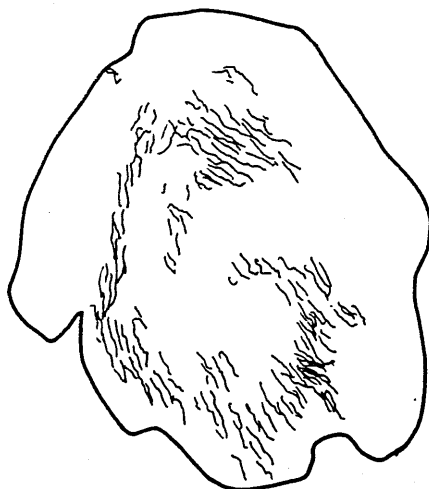
FIGURE 84

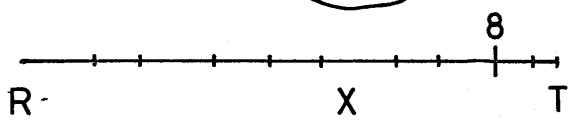
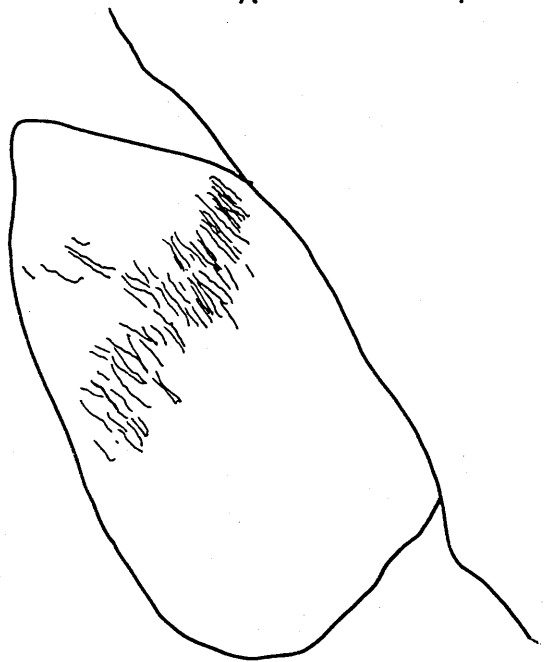
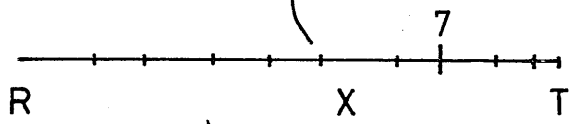
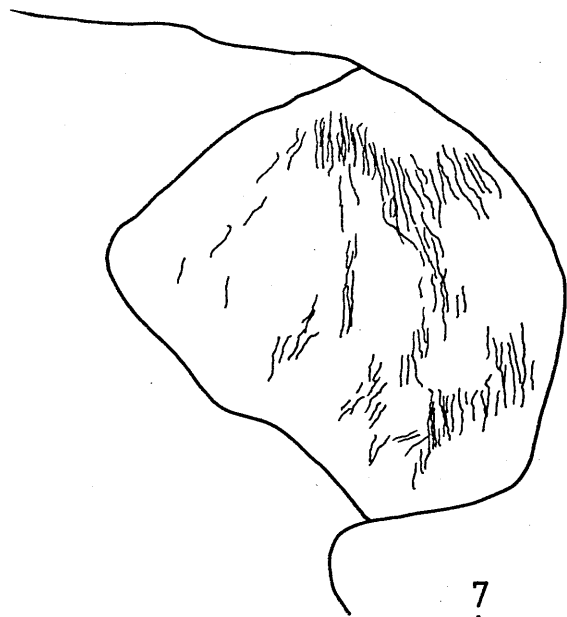


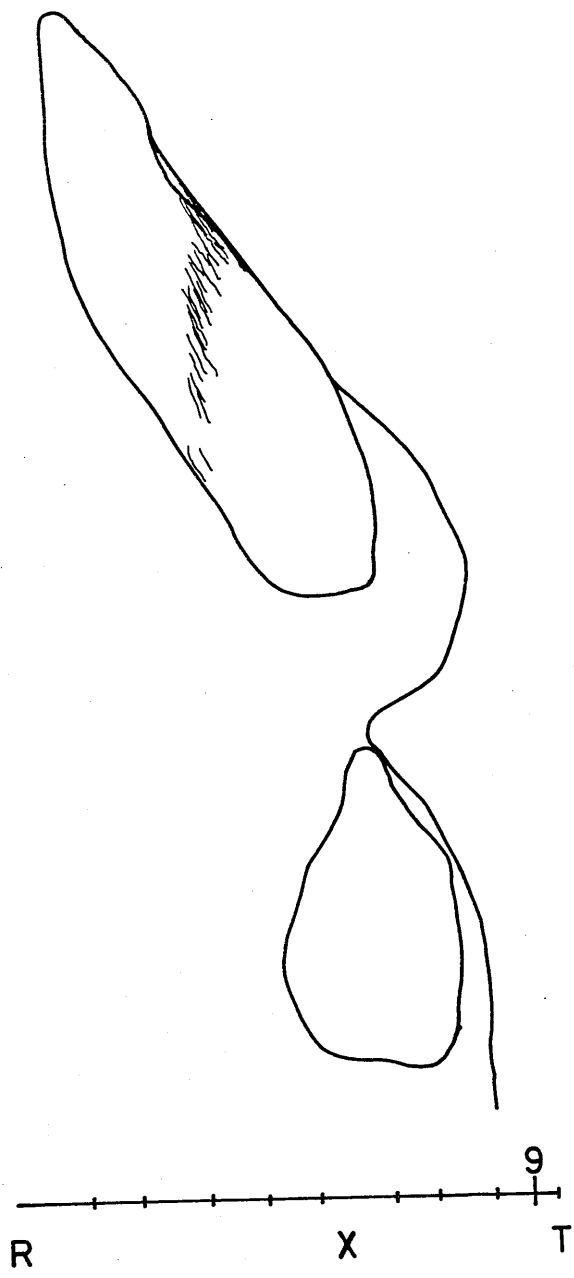
NORMAL OPTIC PATHWAY : VENTRAL SECTOR 1

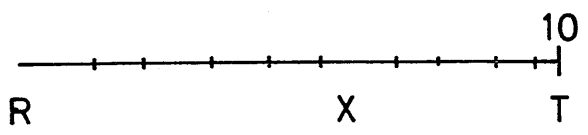
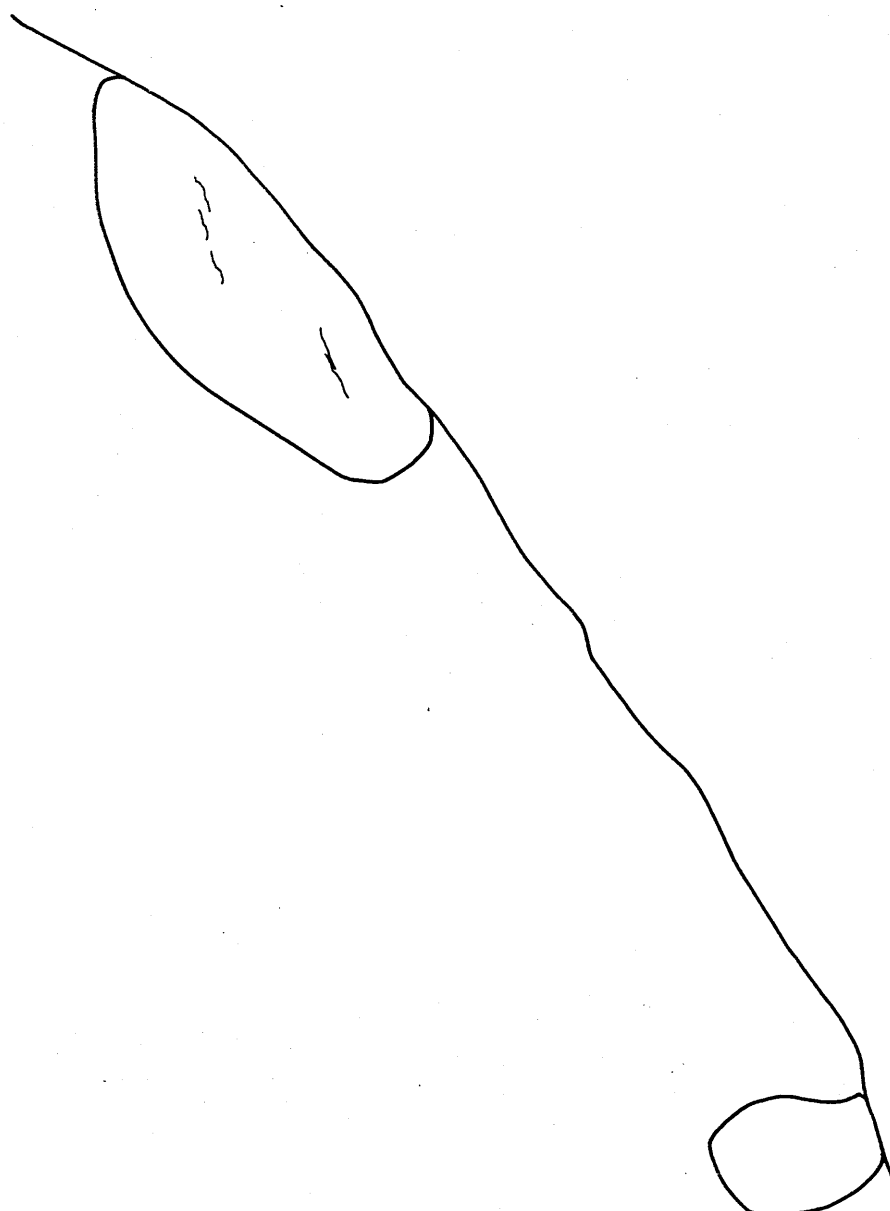




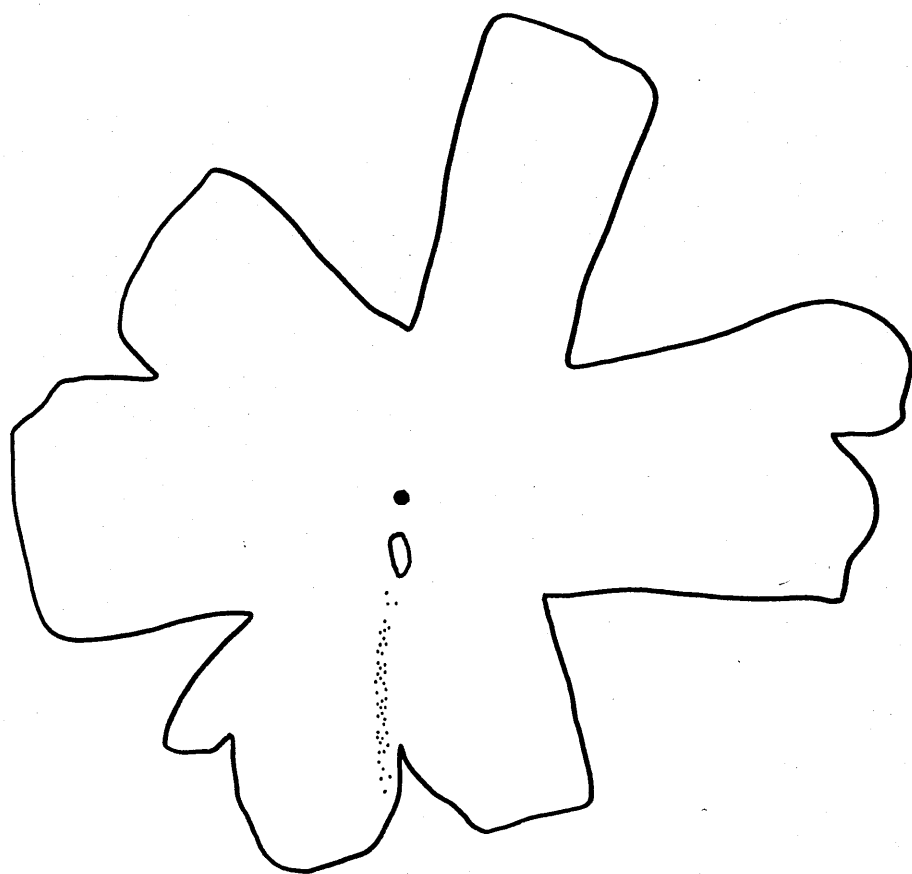








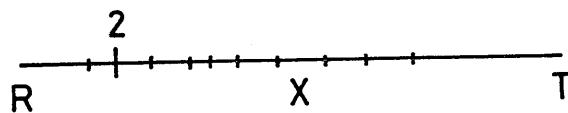
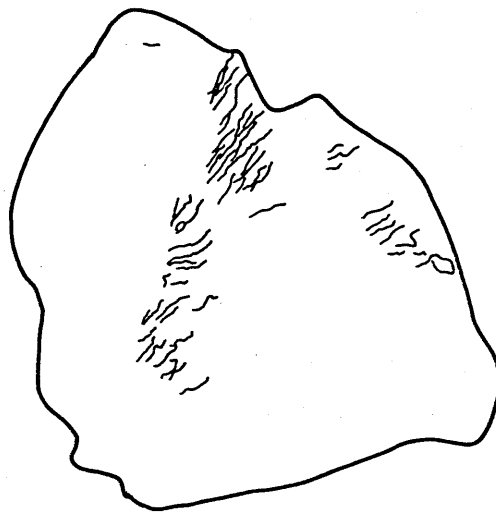
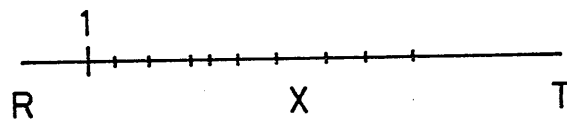
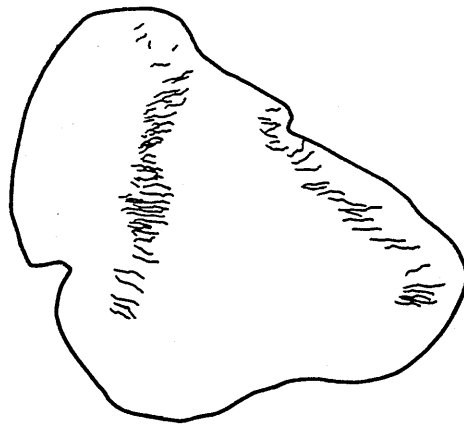


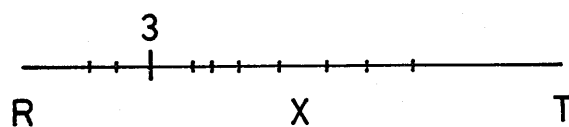


VENTRAL SECTOR

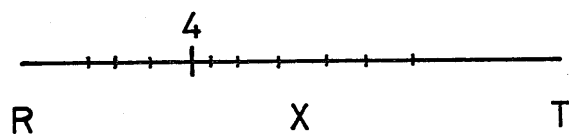
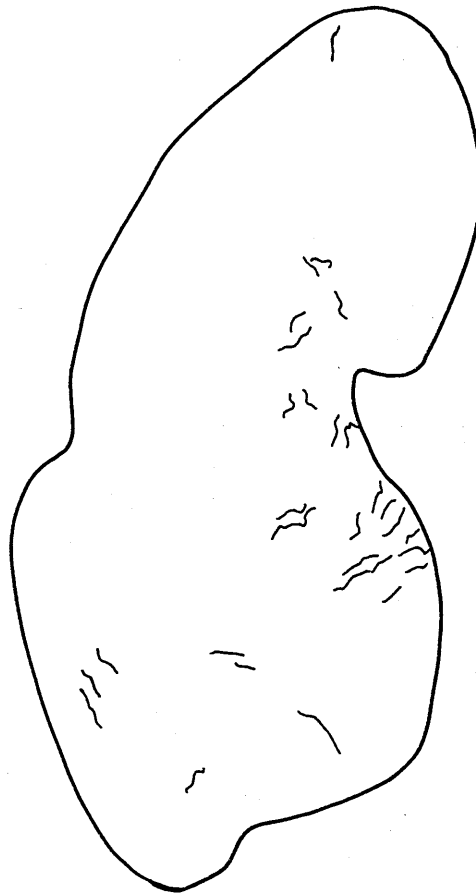
FIGURE 85

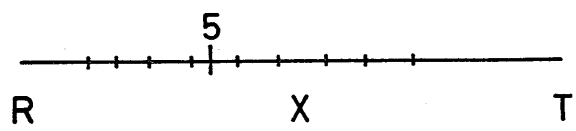
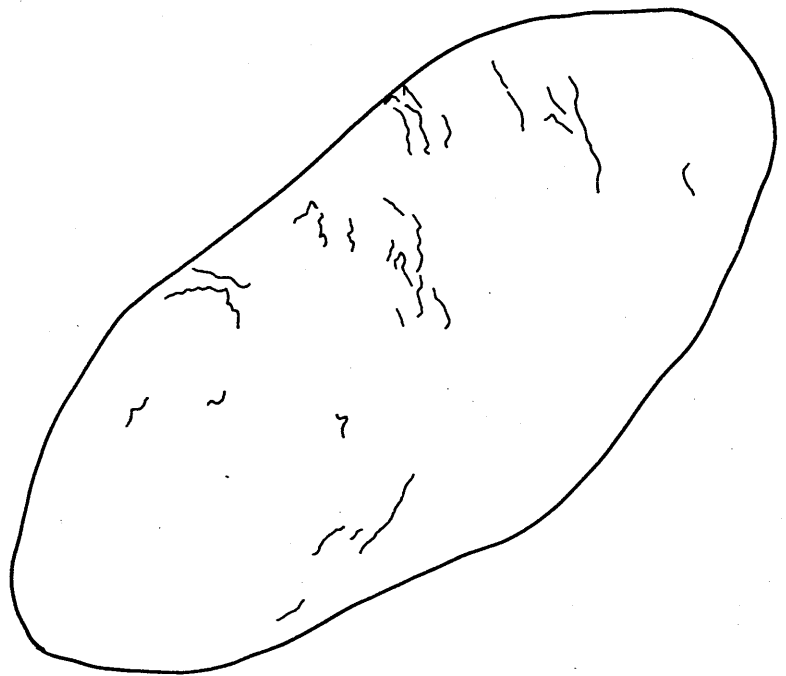
NORMAL OPTIC PATHWAY VENTRAL SECTOR 2

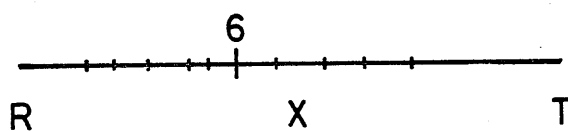
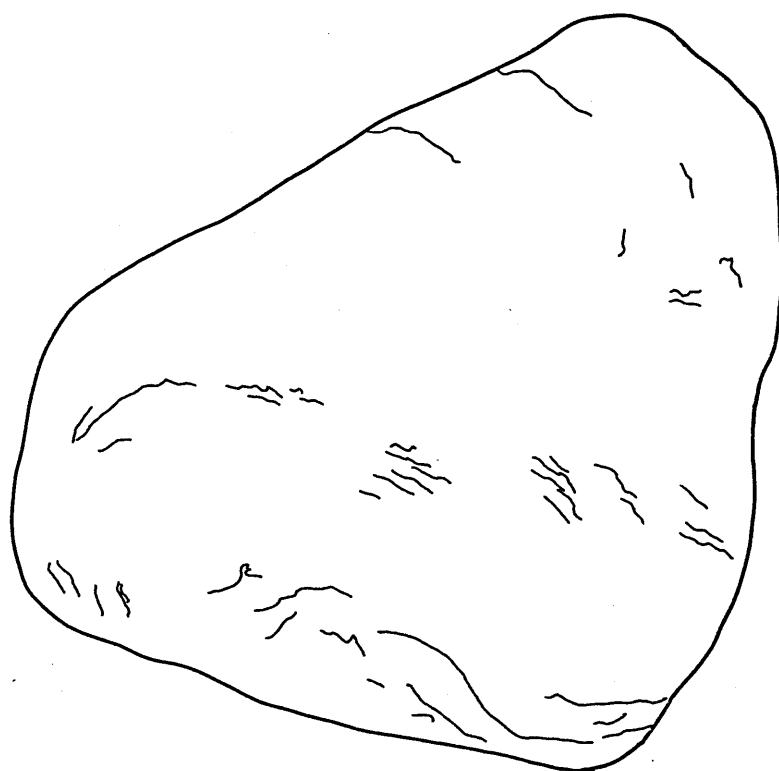




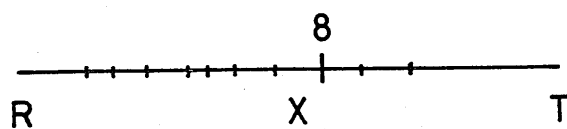
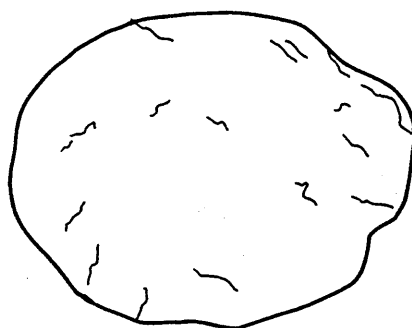
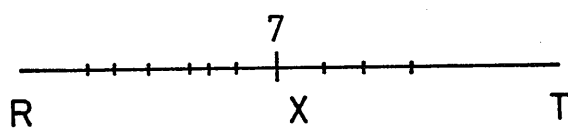
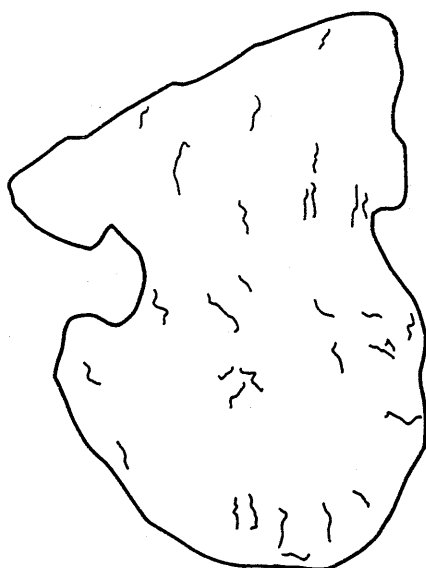
[ 85 ]

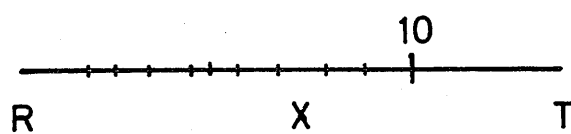
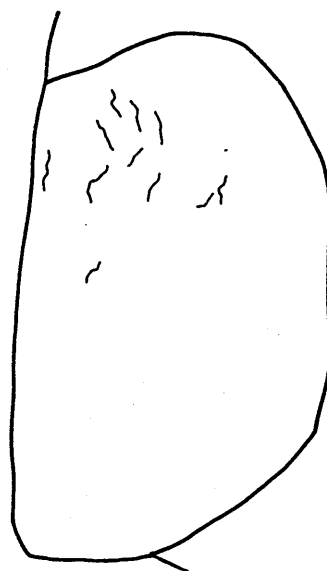
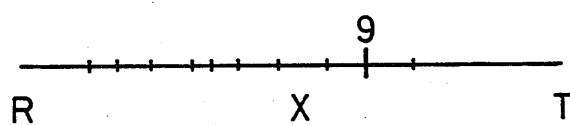
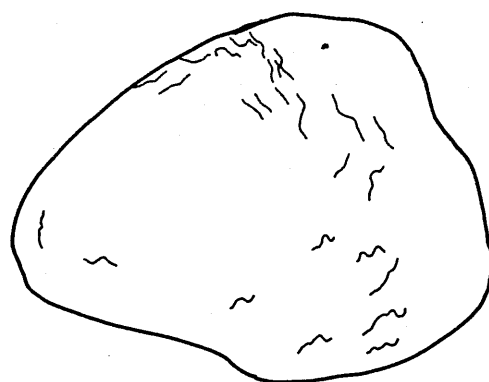




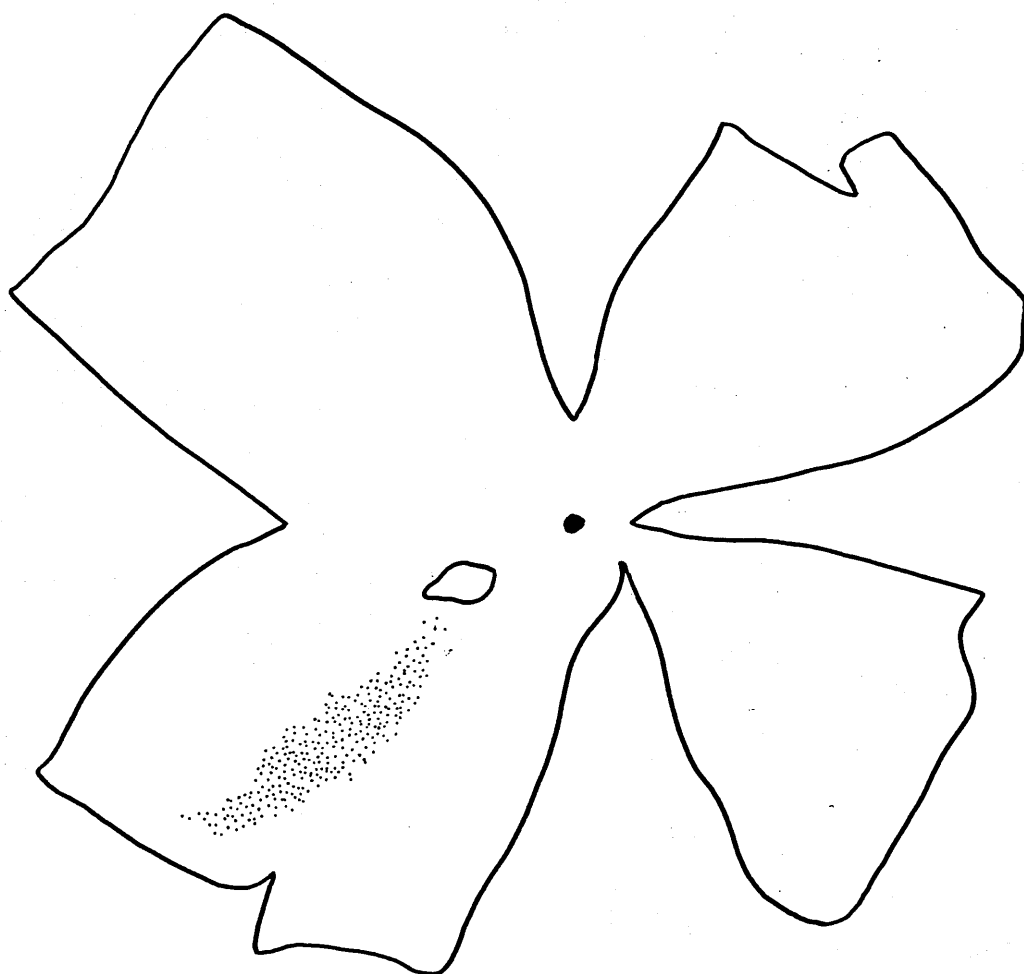


[ 85 ]





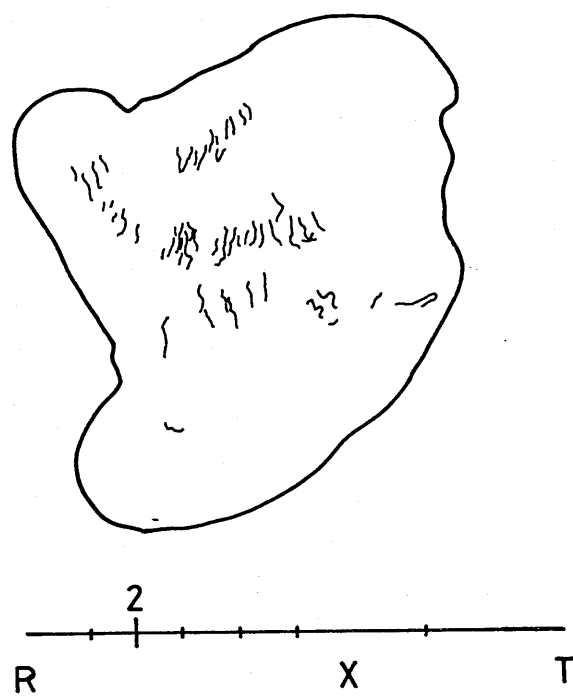
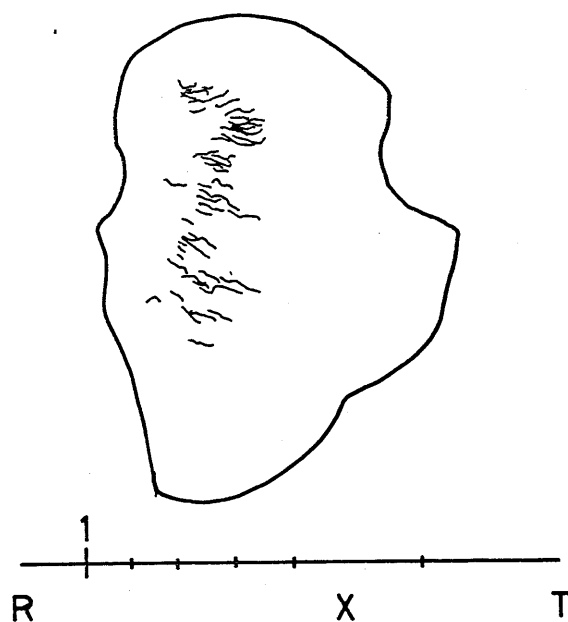


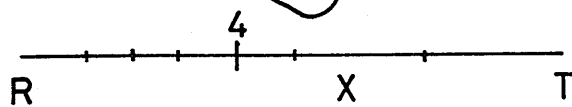
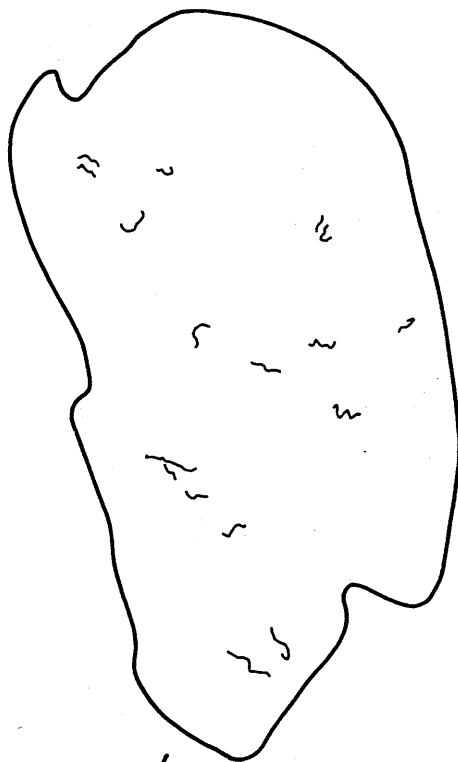
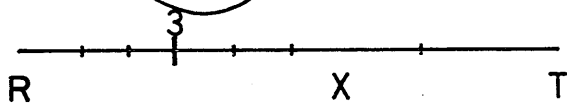
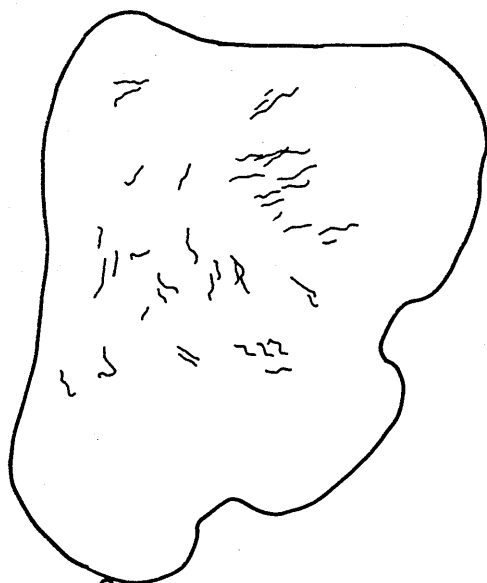


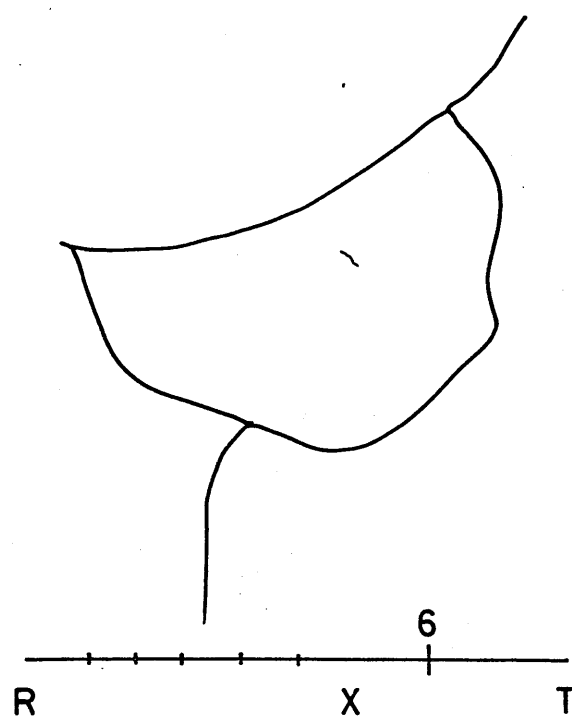
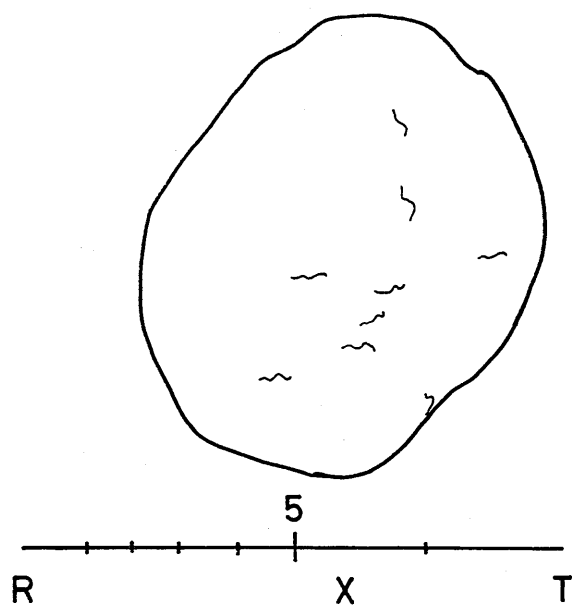
VENTRO - TEMPORAL SECTOR

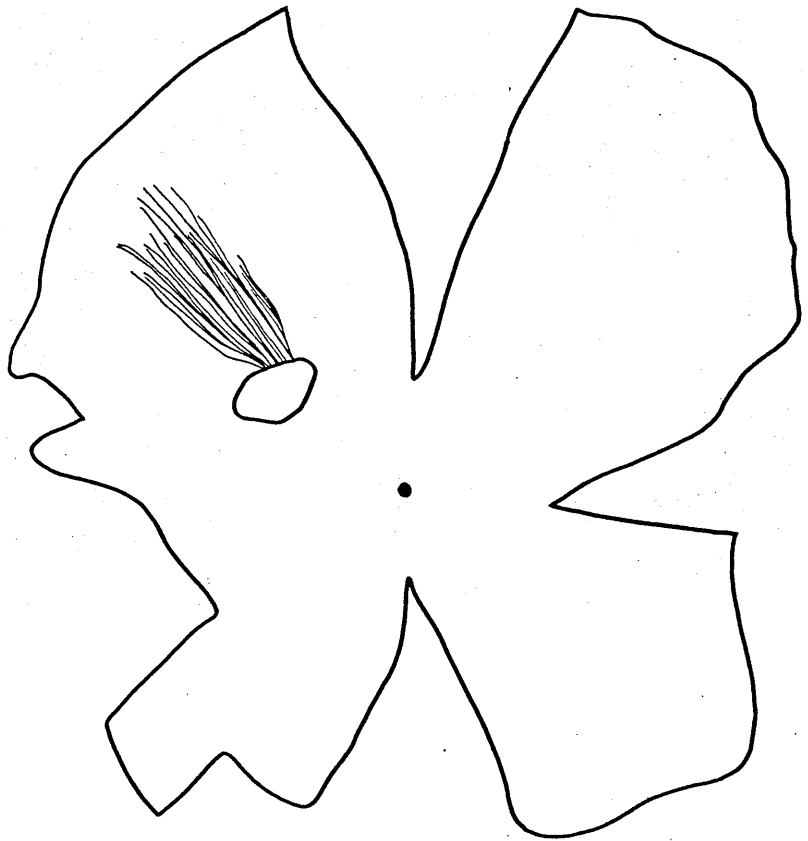
FIGURE 86

VENTRO -  
NORMAL OPTIC PATHWAY : TEMPORAL SECTOR







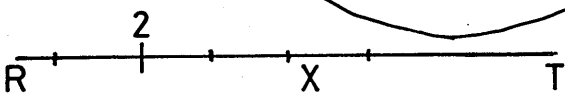
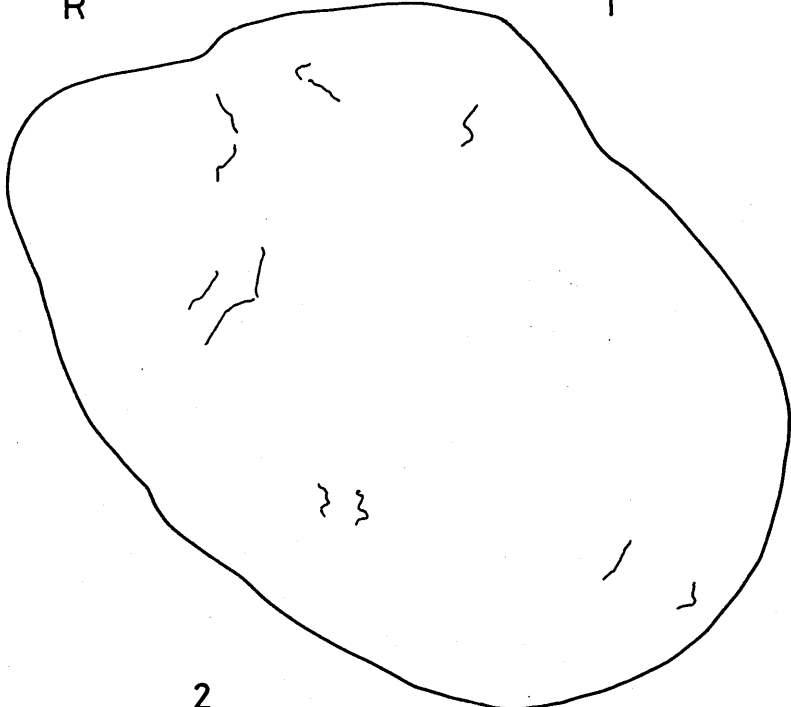
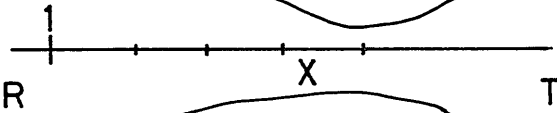
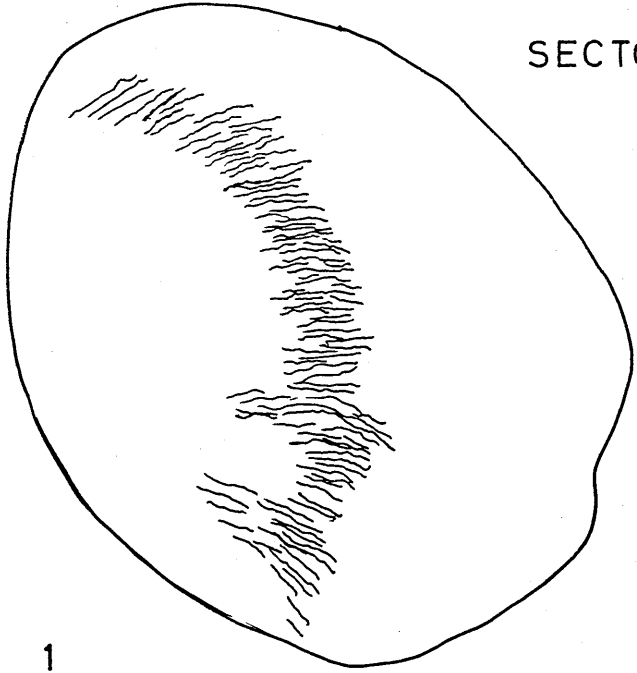


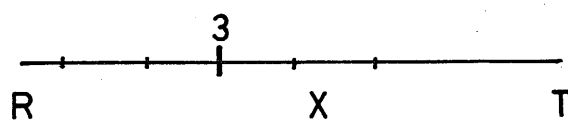
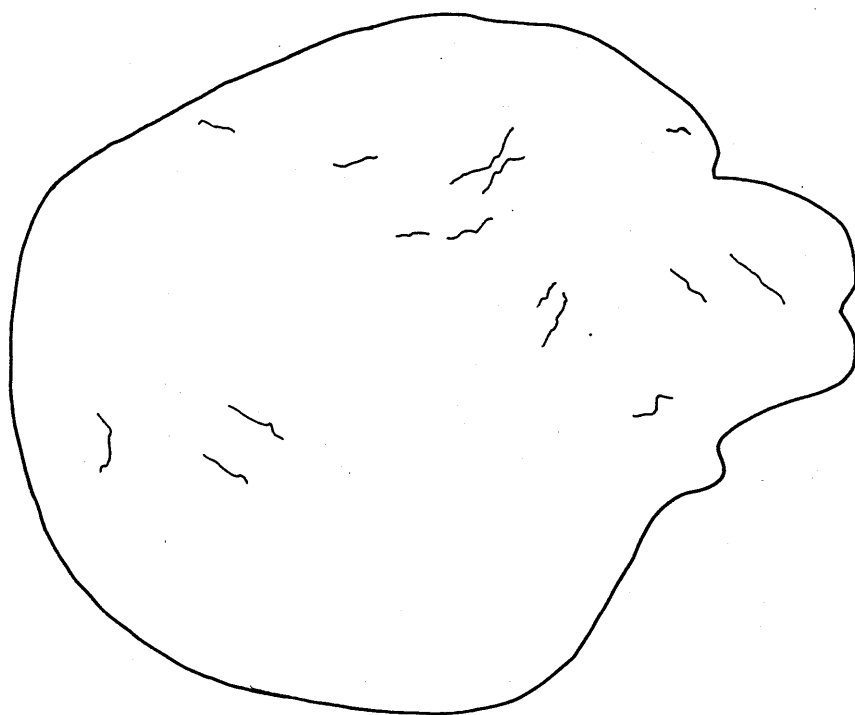
DORSO -TEMPORAL  
SECTOR

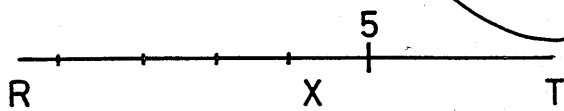
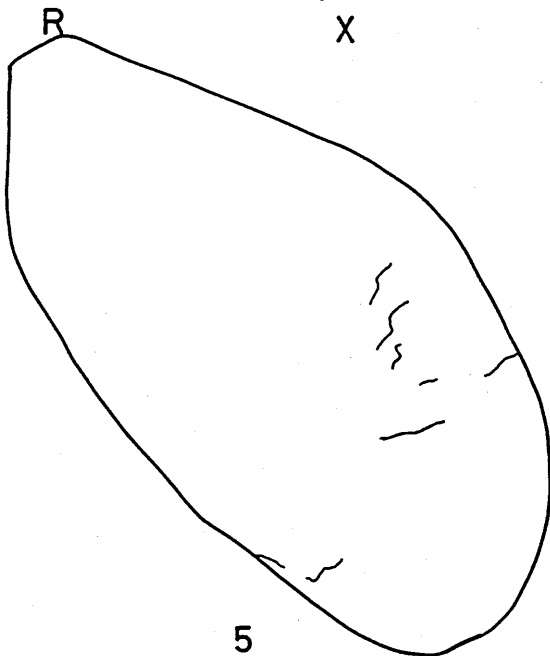
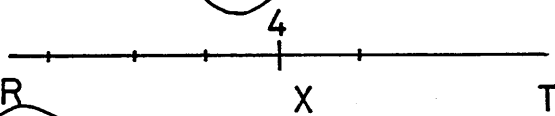
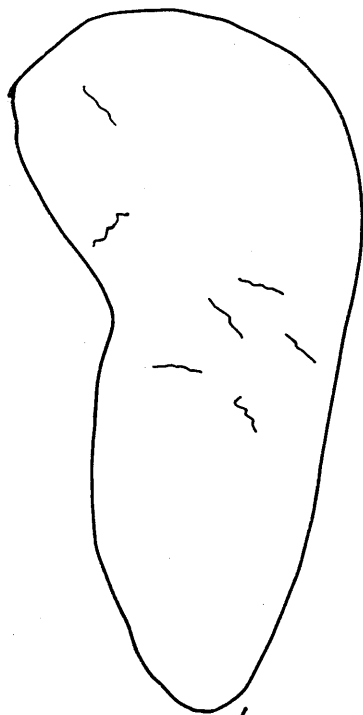
FIGURE 87

REGENERATED PATHWAY : DORSO-TEMPORAL

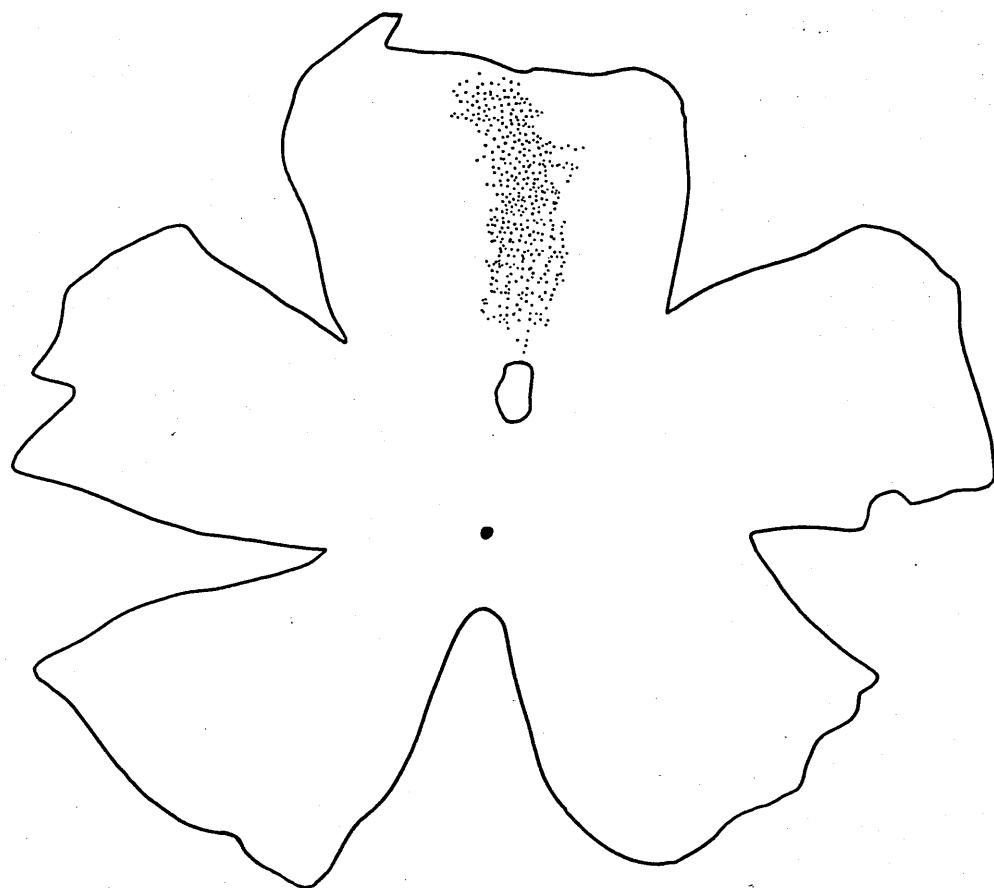
SECTOR







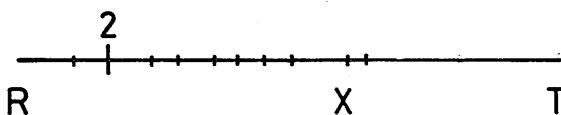
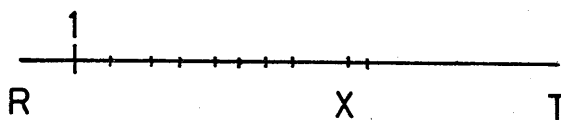
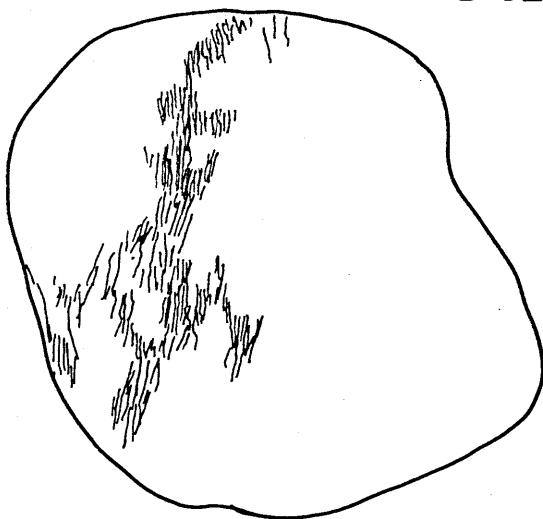


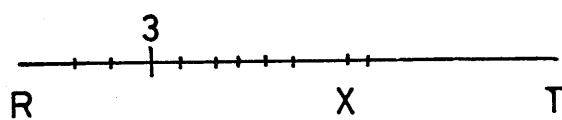
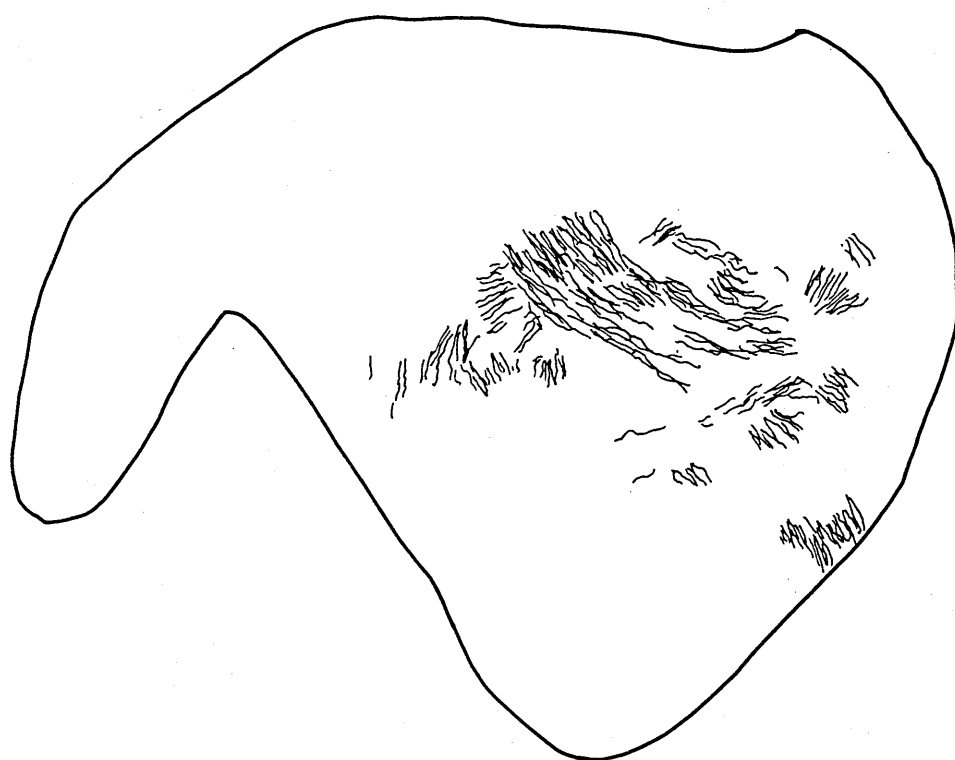


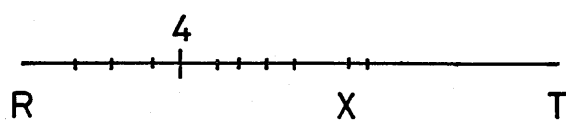
DORSAL SECTOR

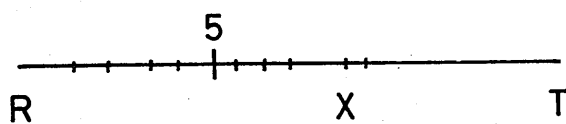
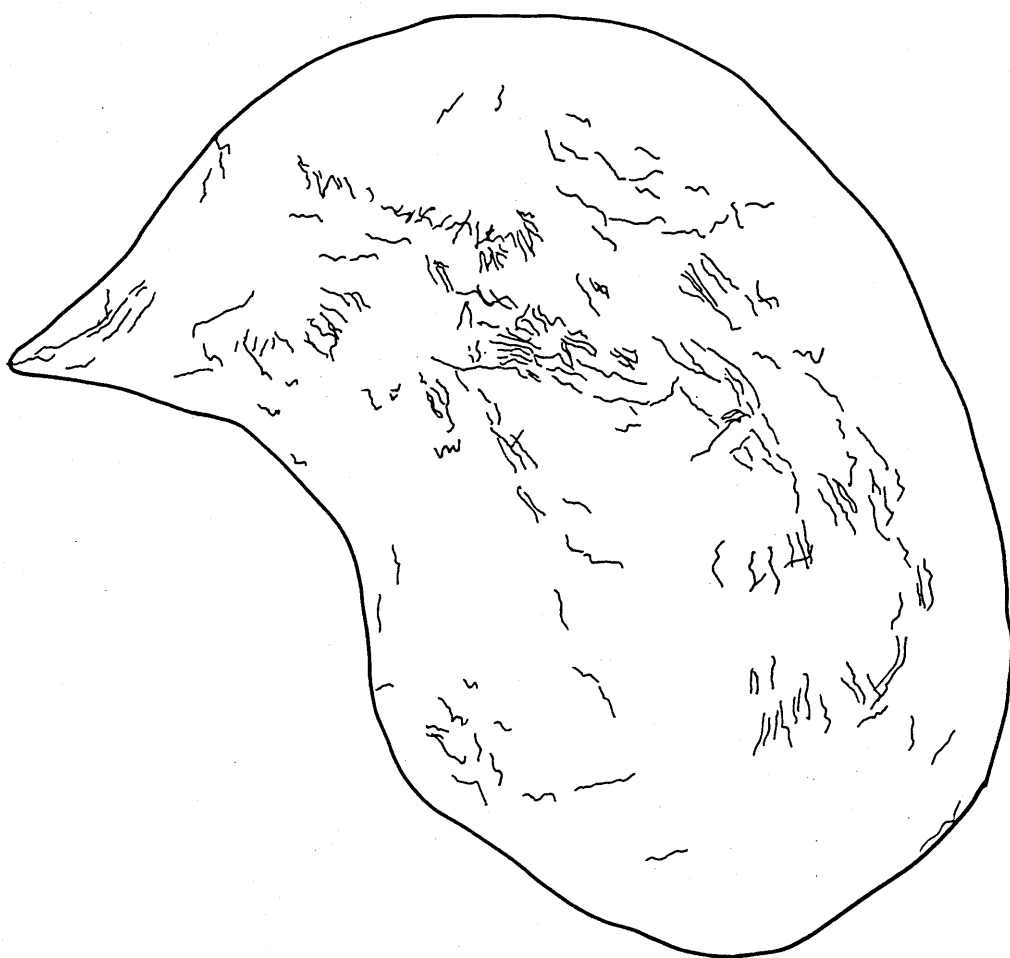
FIGURE 88

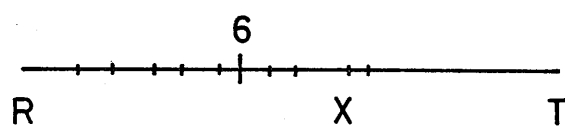
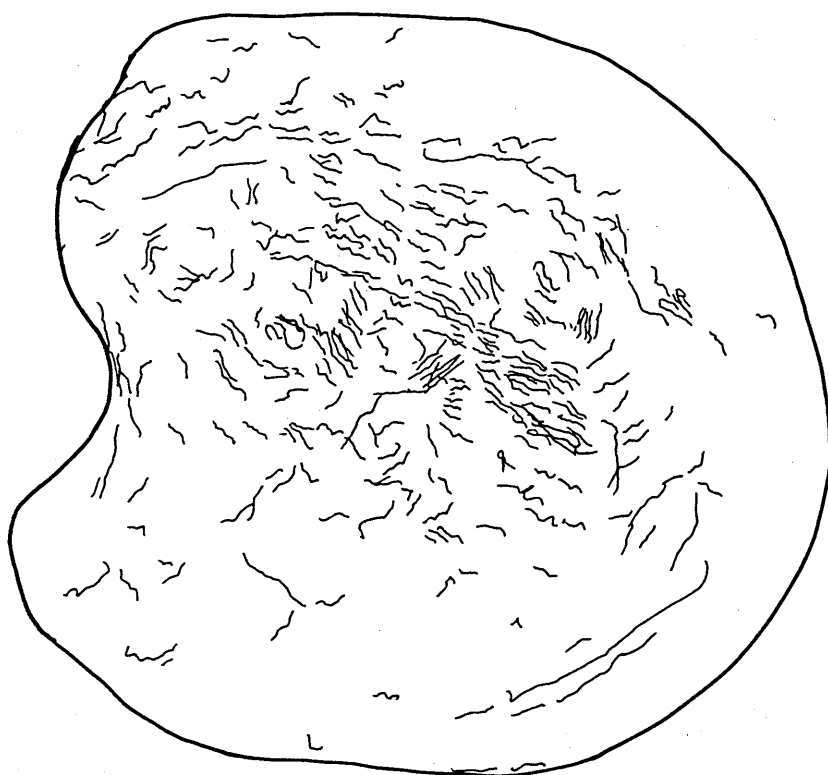
REGENERATED PATHWAY : DORSAL SECTOR

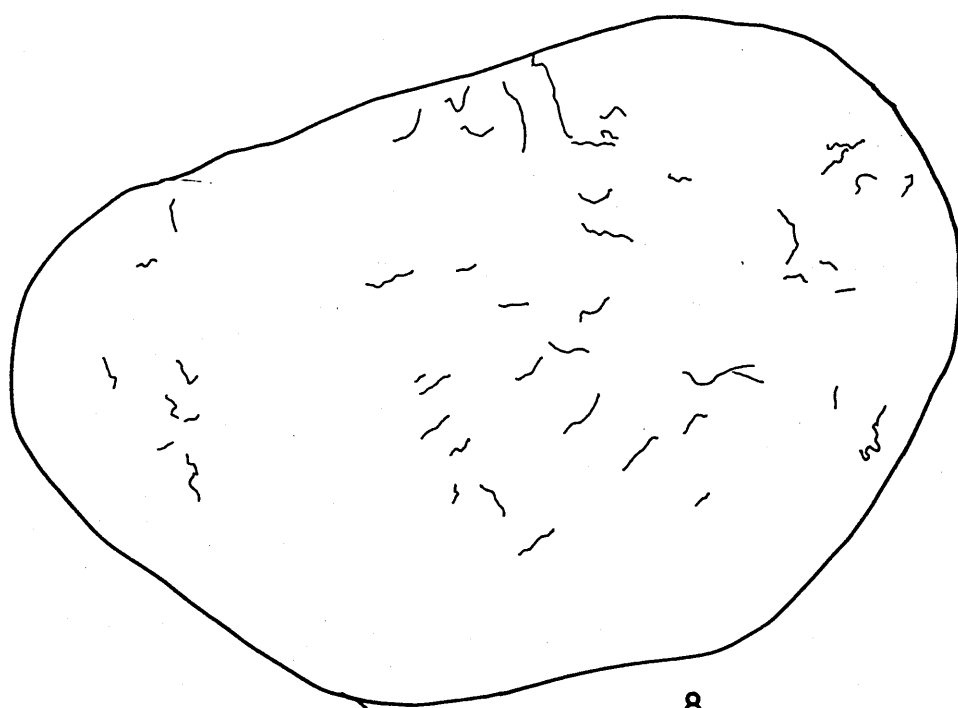
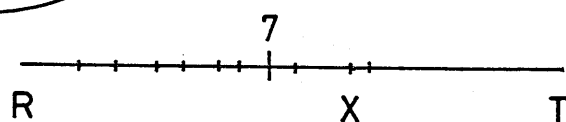




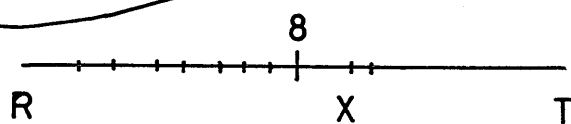


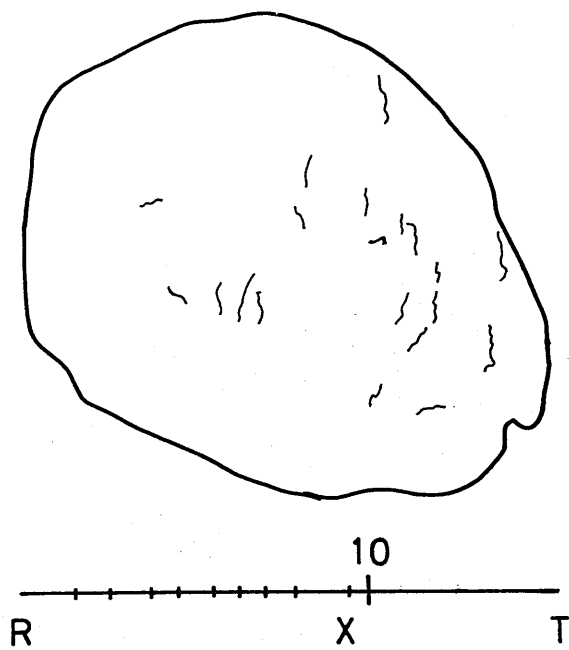
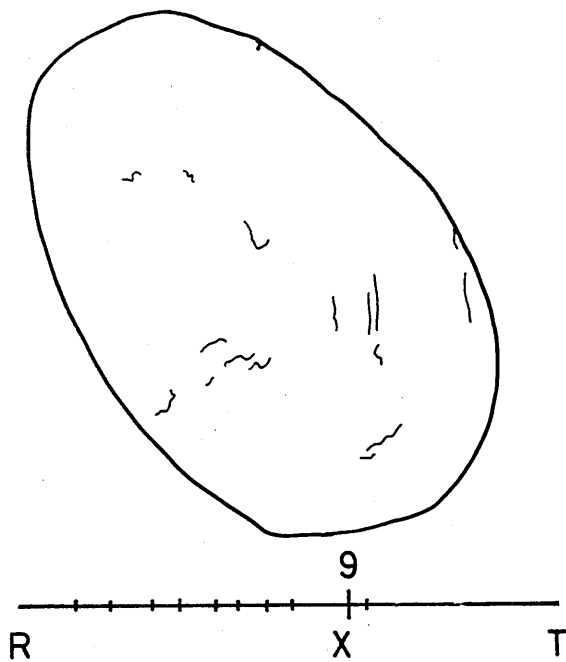




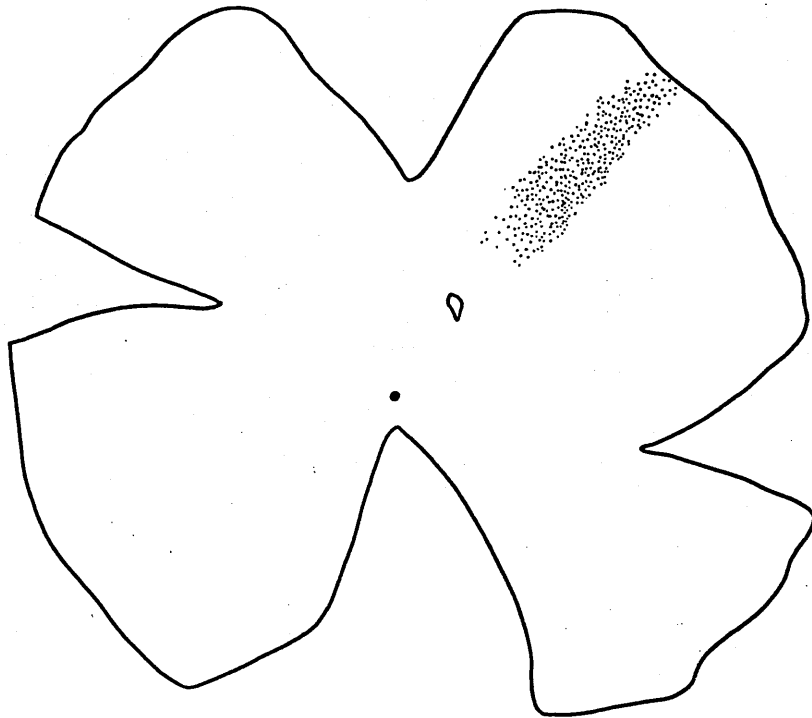


[88]





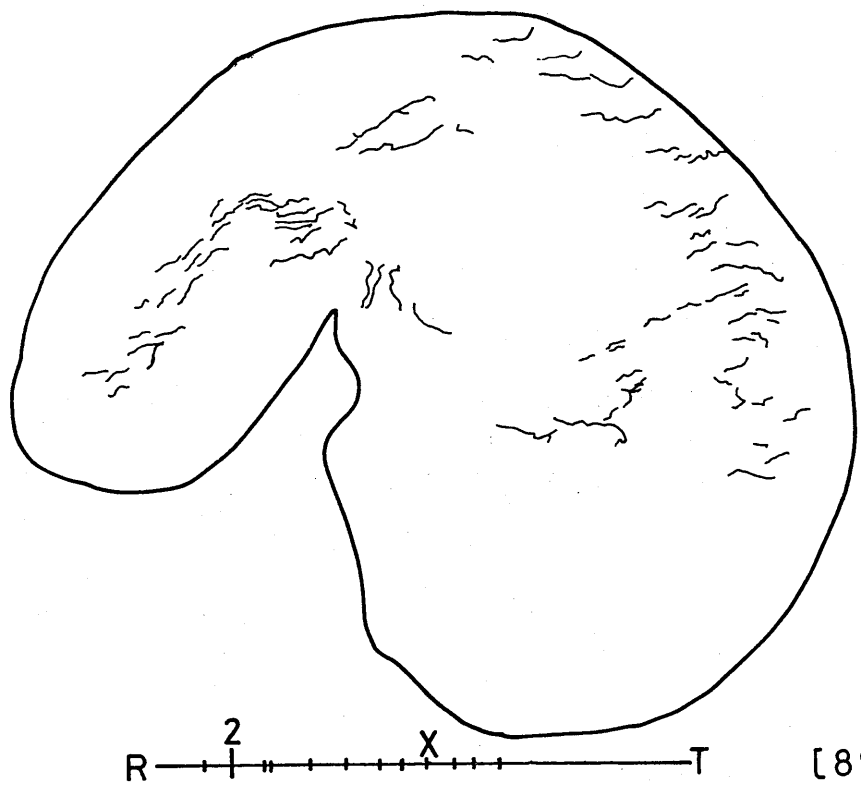
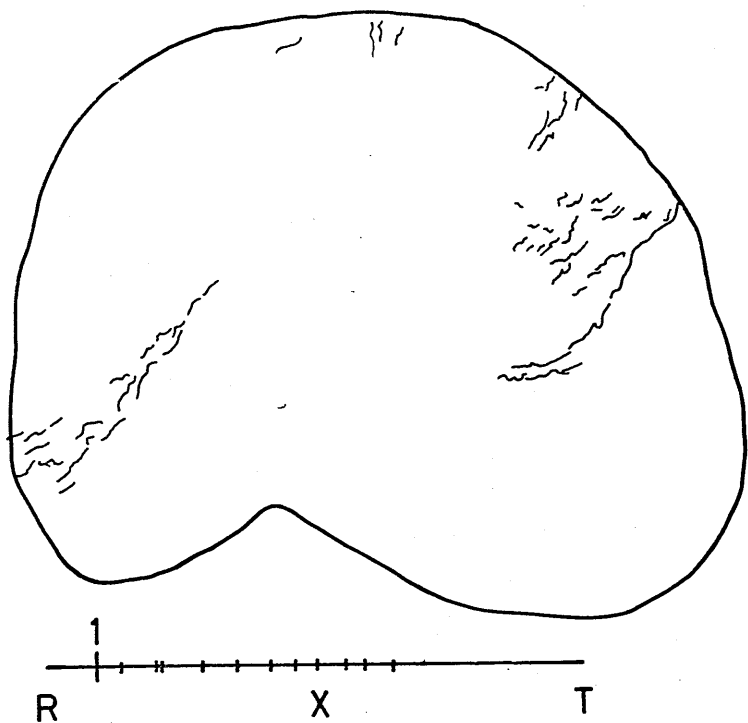


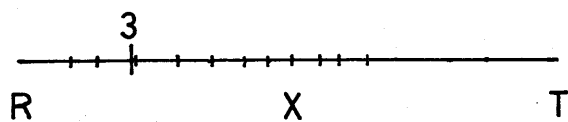
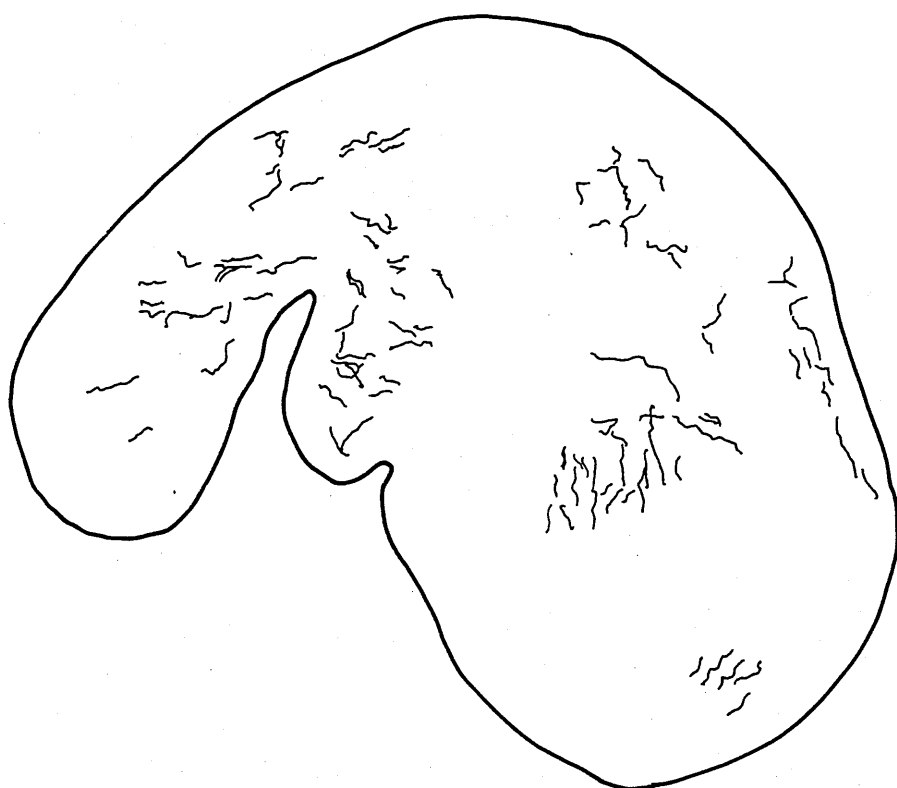


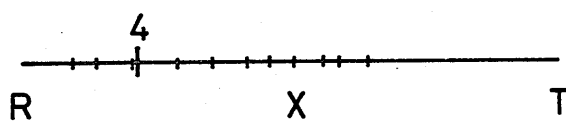
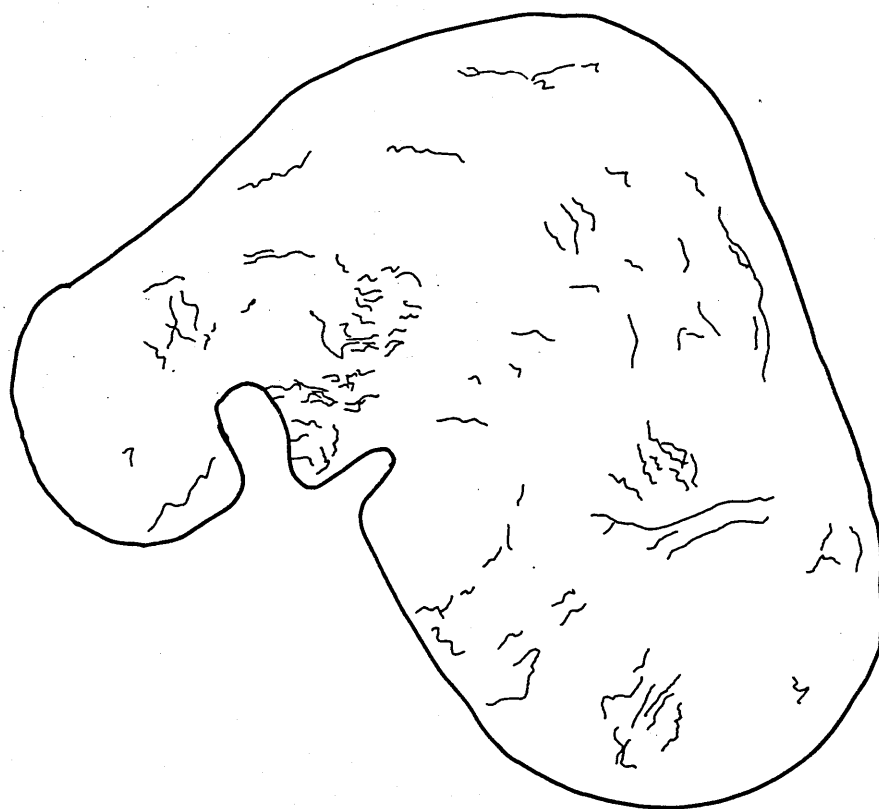
DORSO-NASAL SECTOR

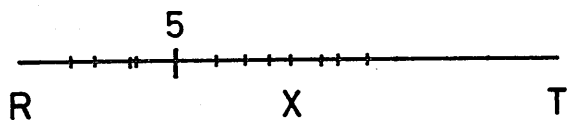
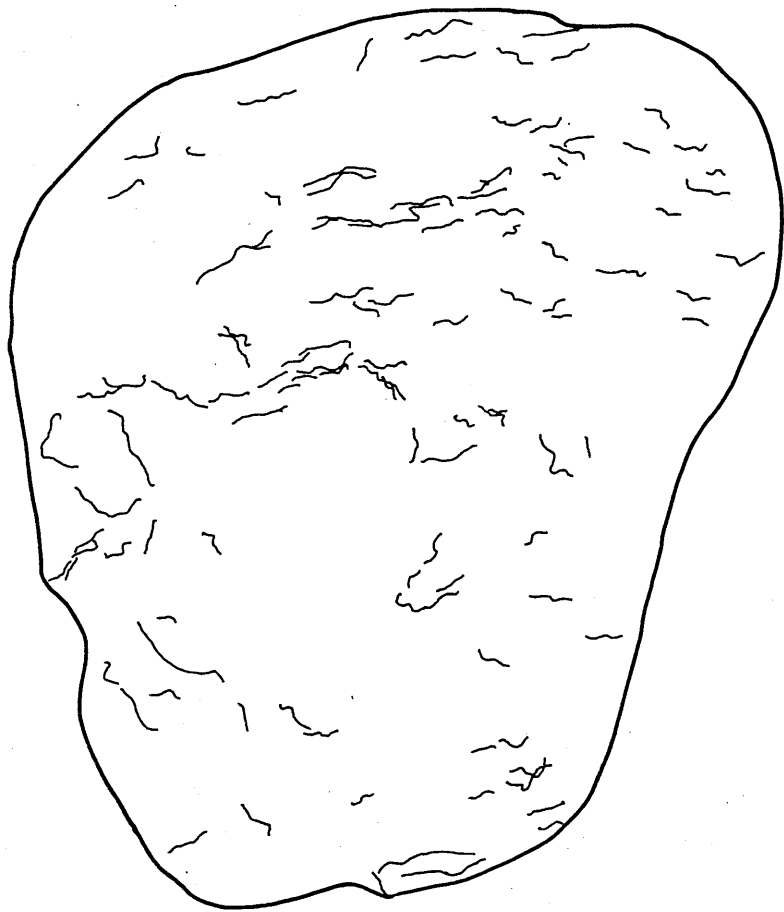
FIGURE 89

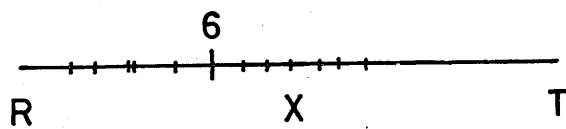
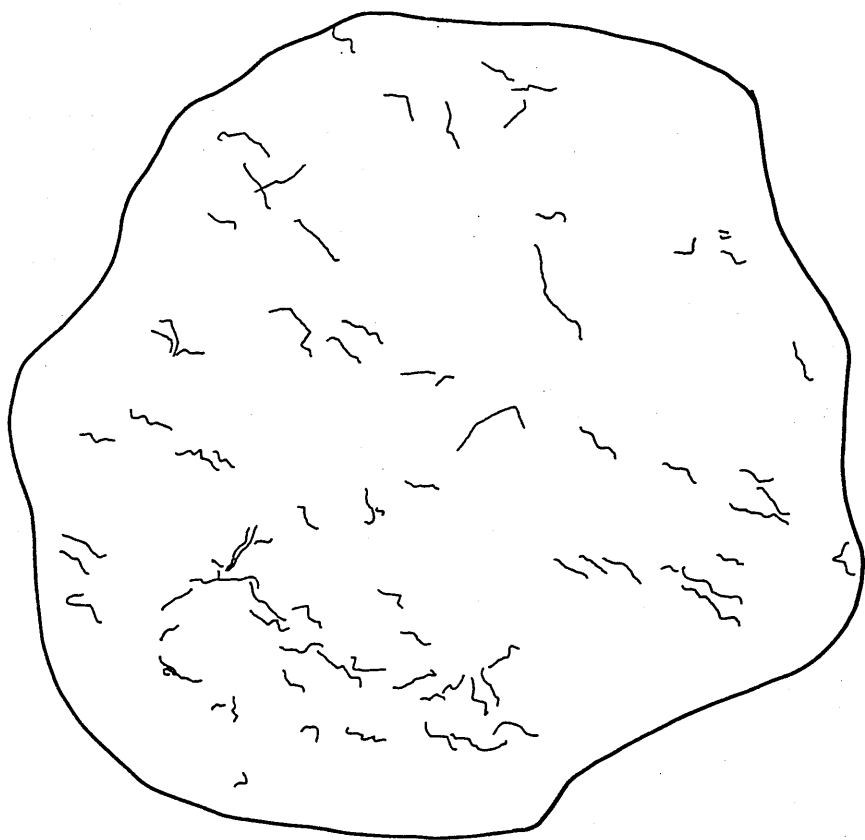
REGENERATED : DORSO-NASAL SECTOR

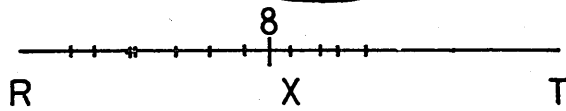
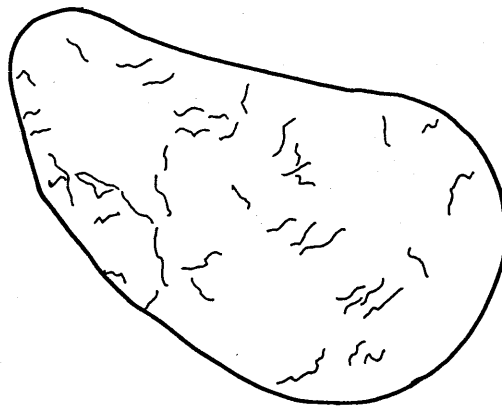
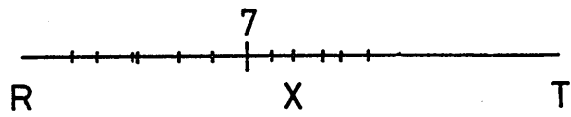
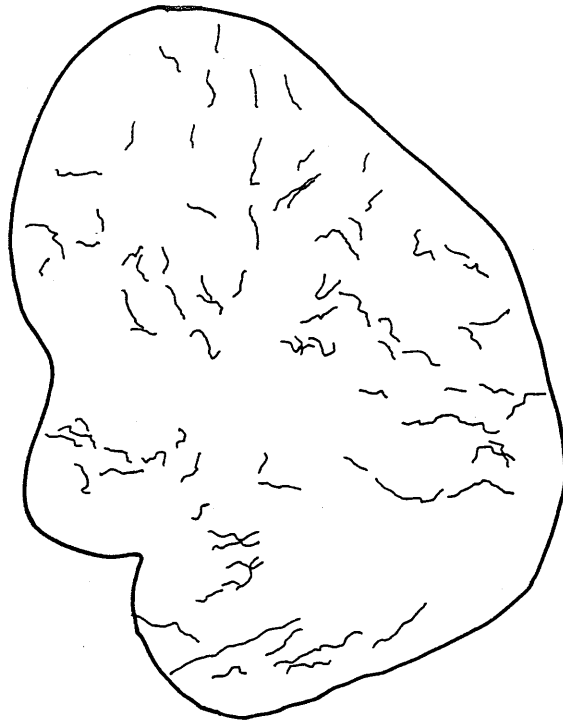


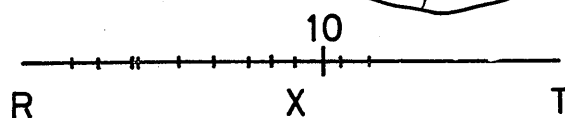
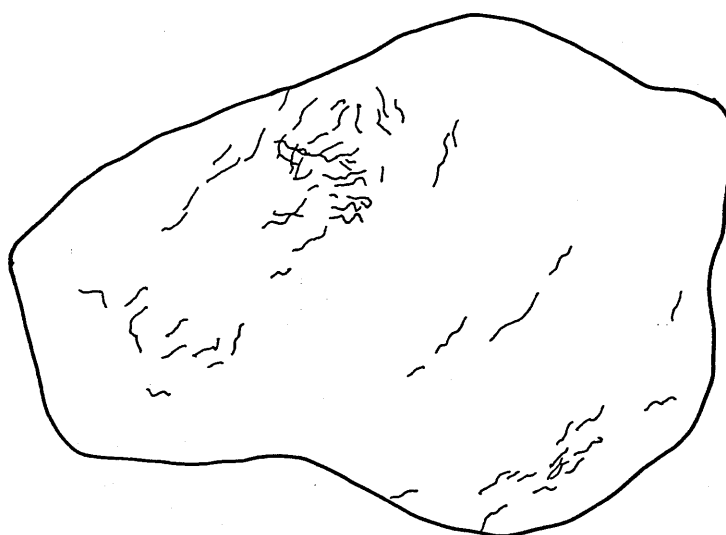
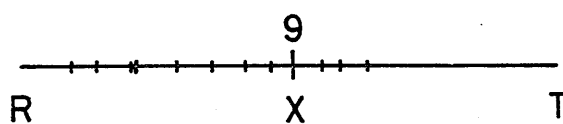
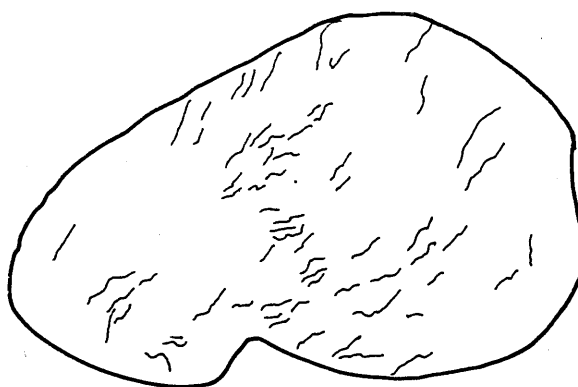




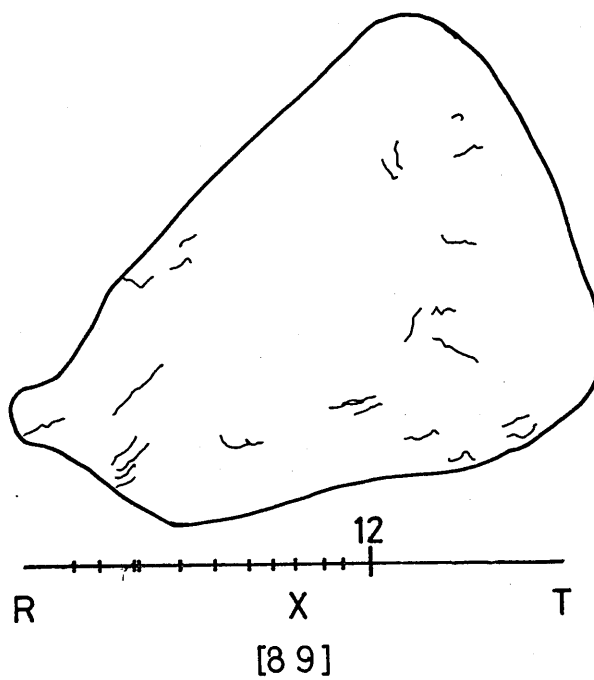
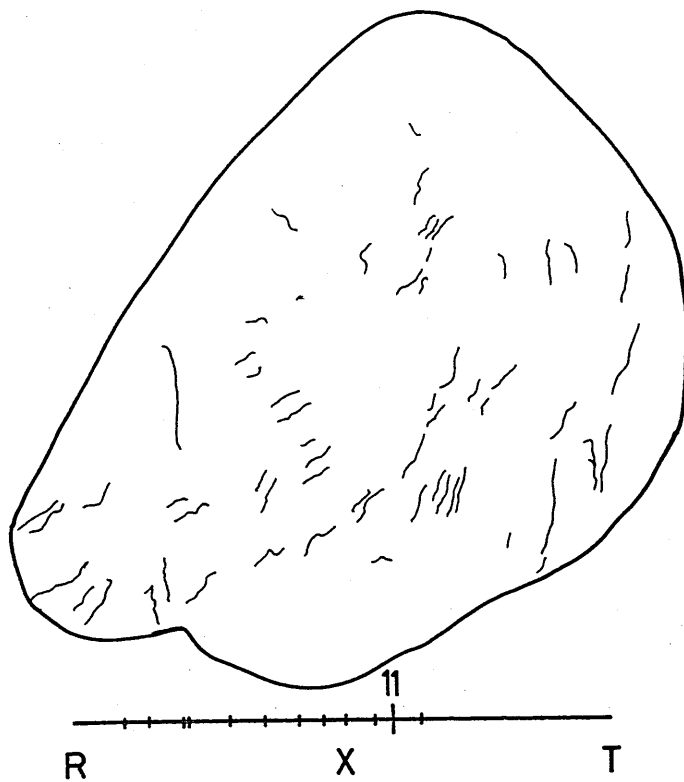


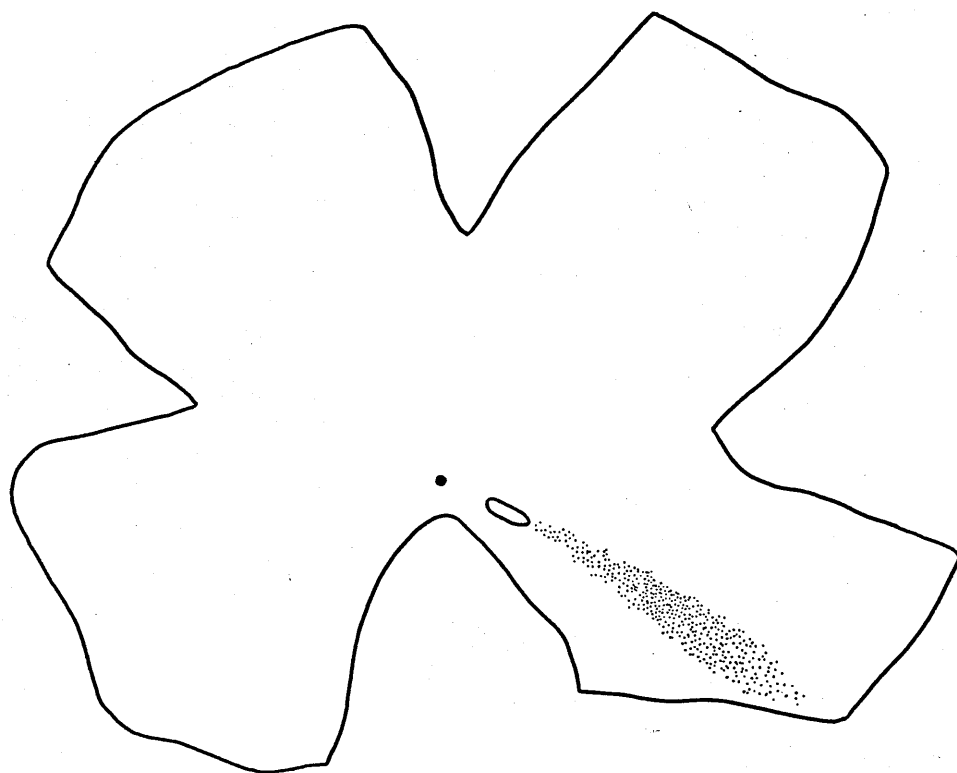










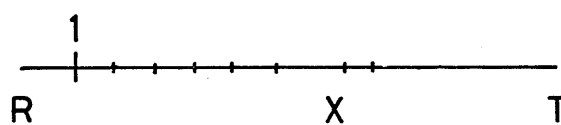
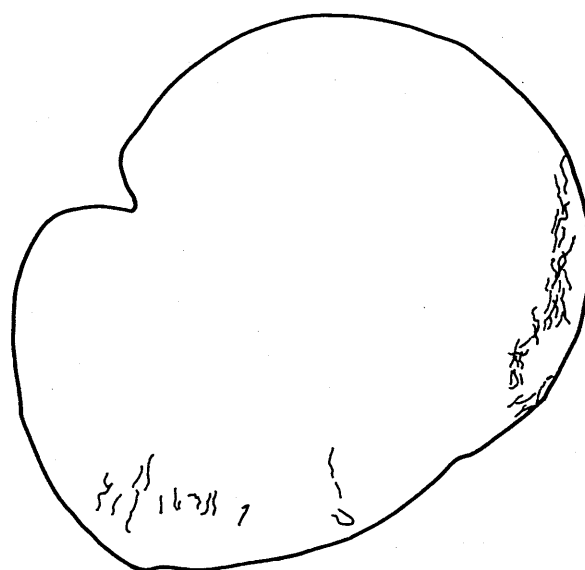


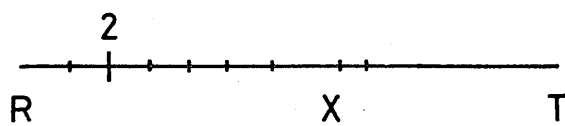
VENTRO - NASAL SECTOR

FIGURE 90

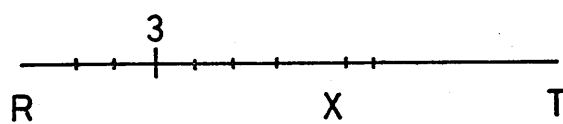
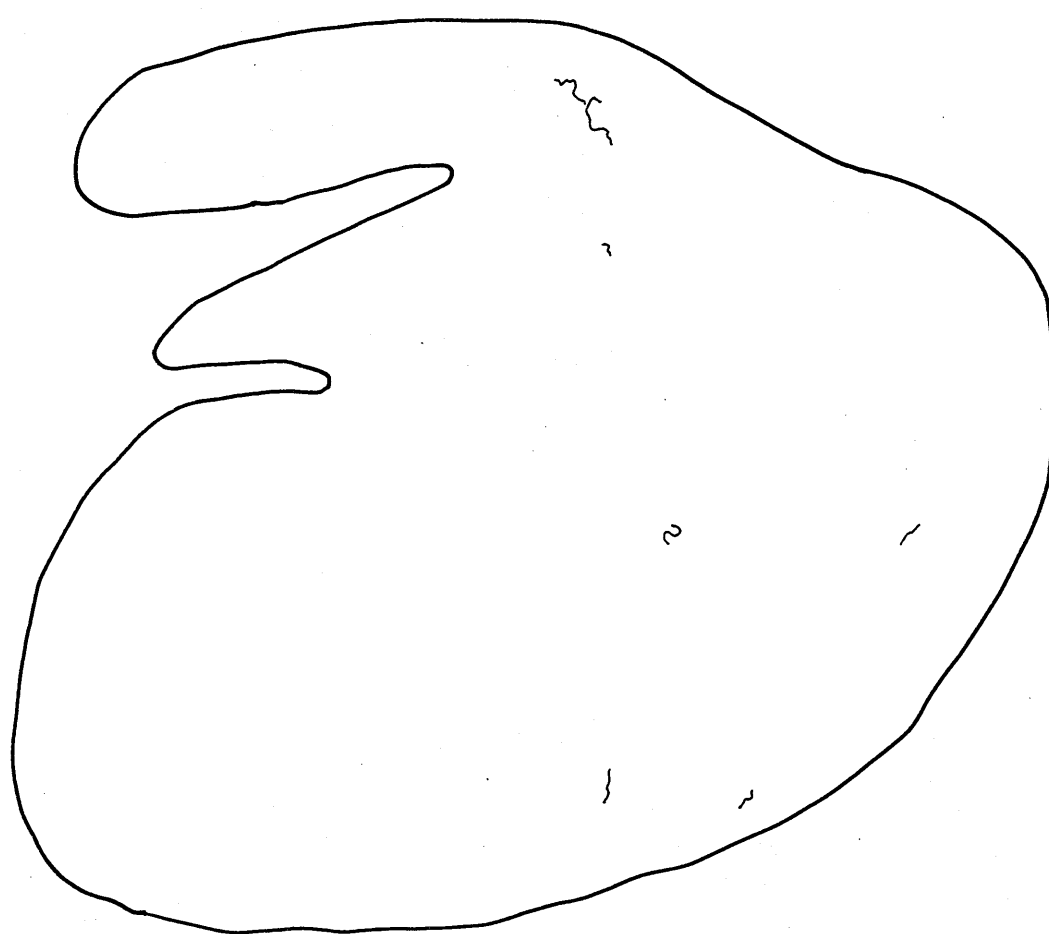
REGENERATED PATHWAY :

VENTRO-NASAL SECTOR

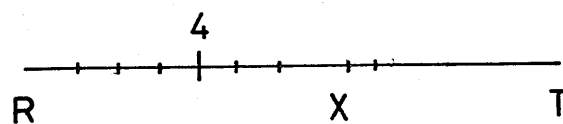
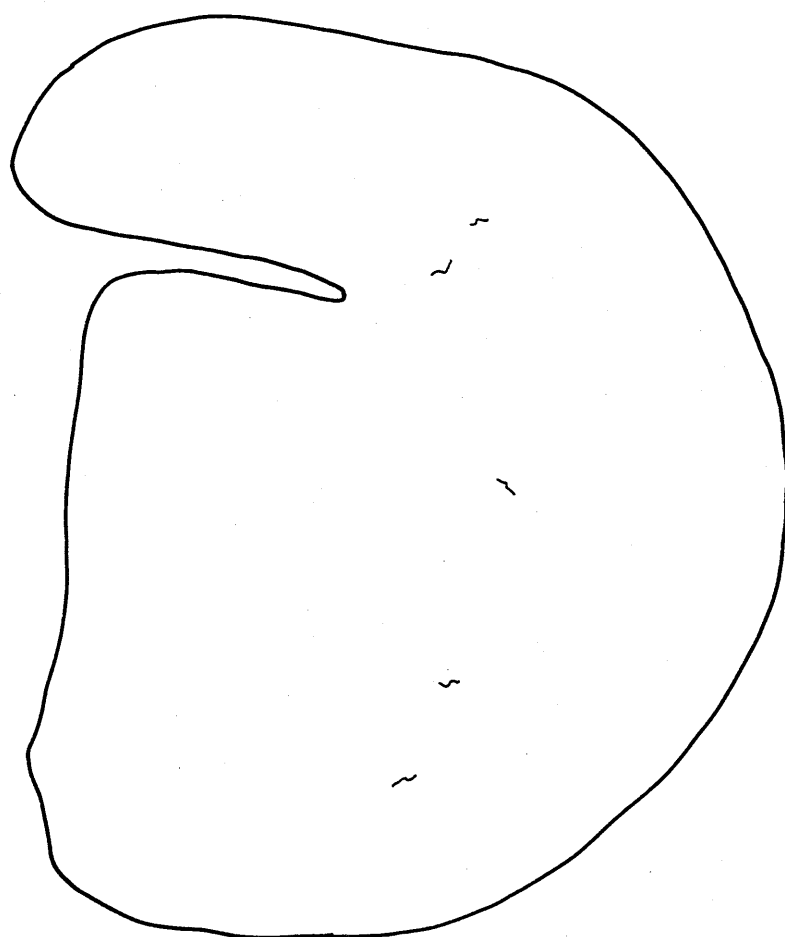




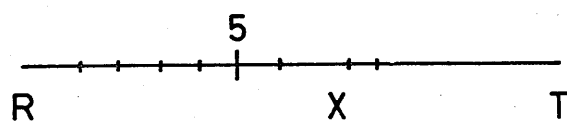
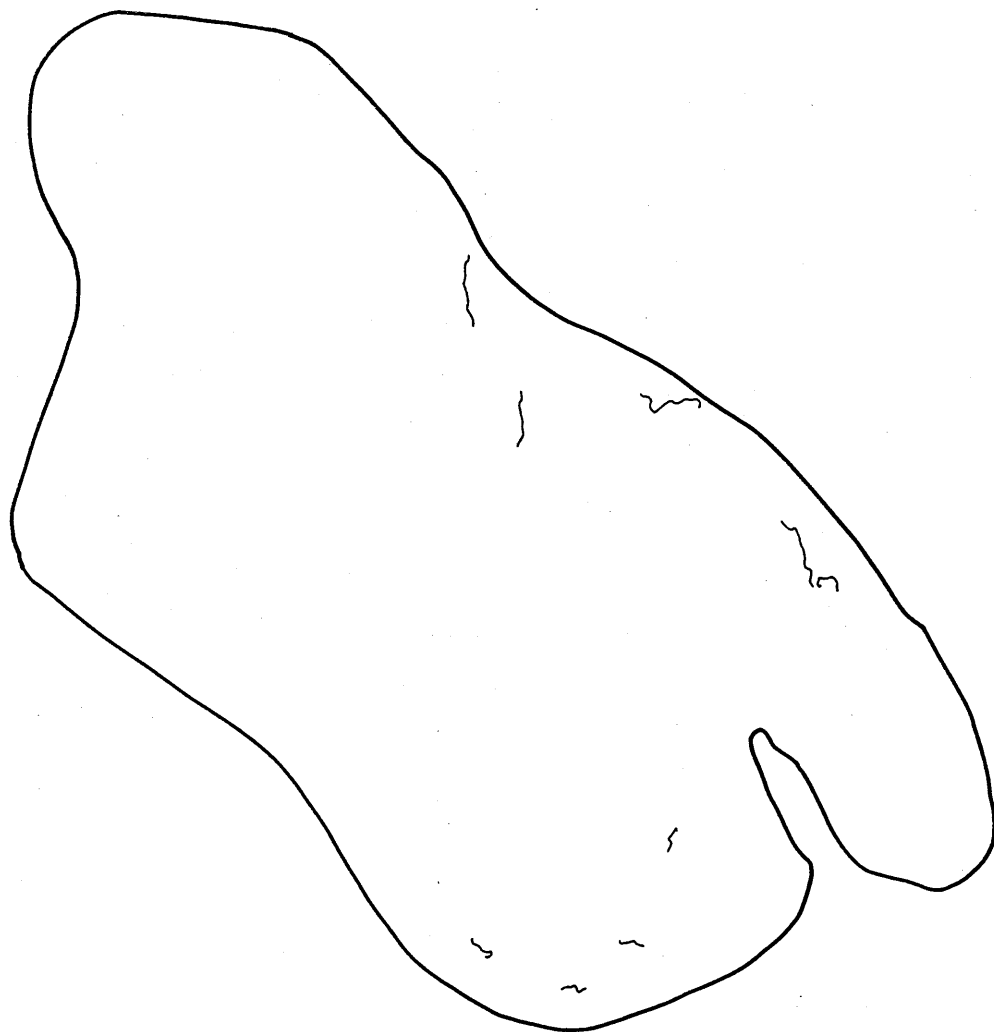
[ 90 ]



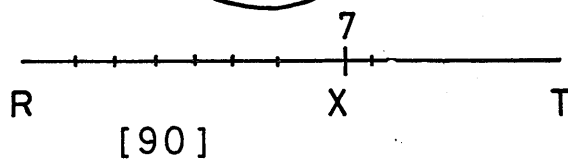
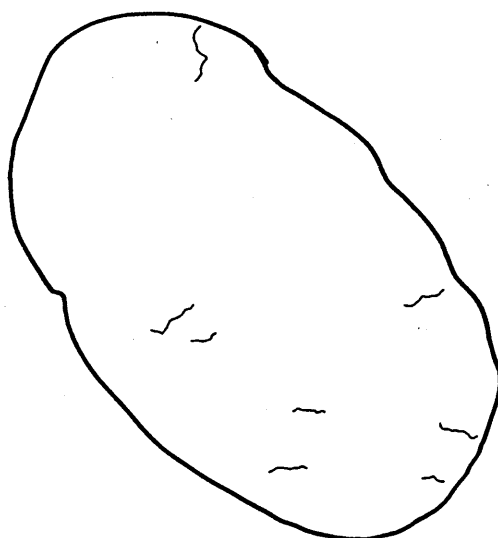
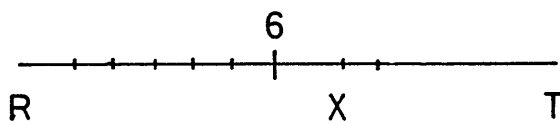
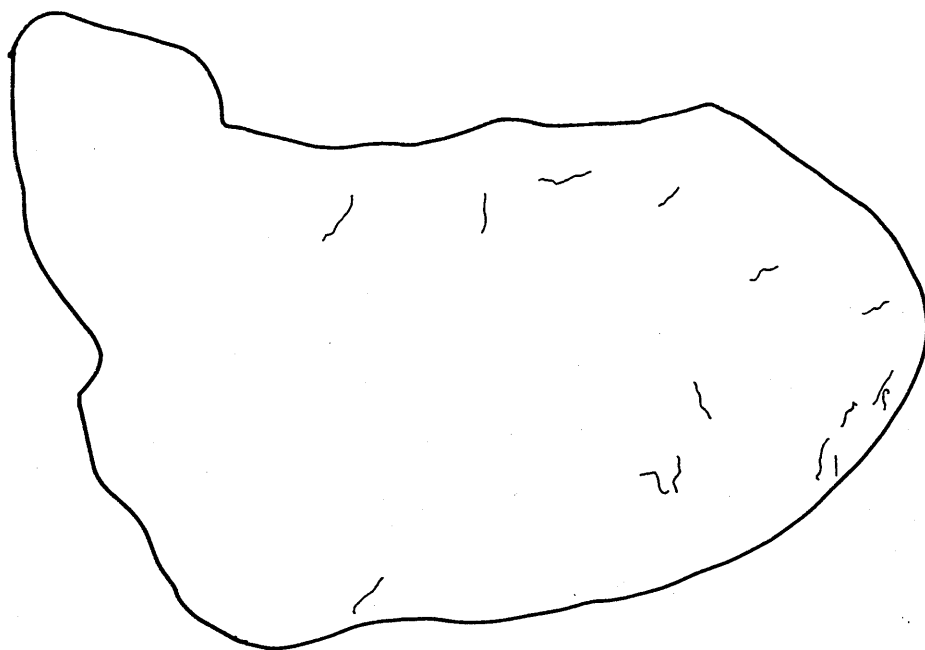
[90]



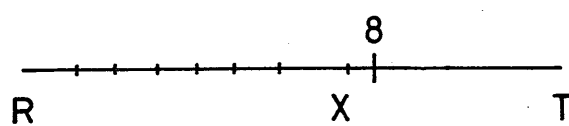
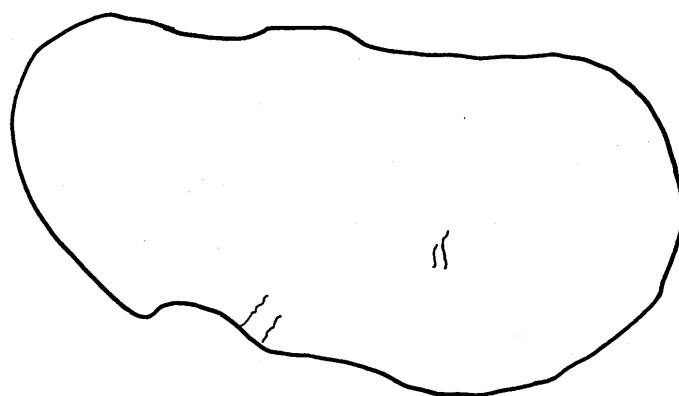
[90]



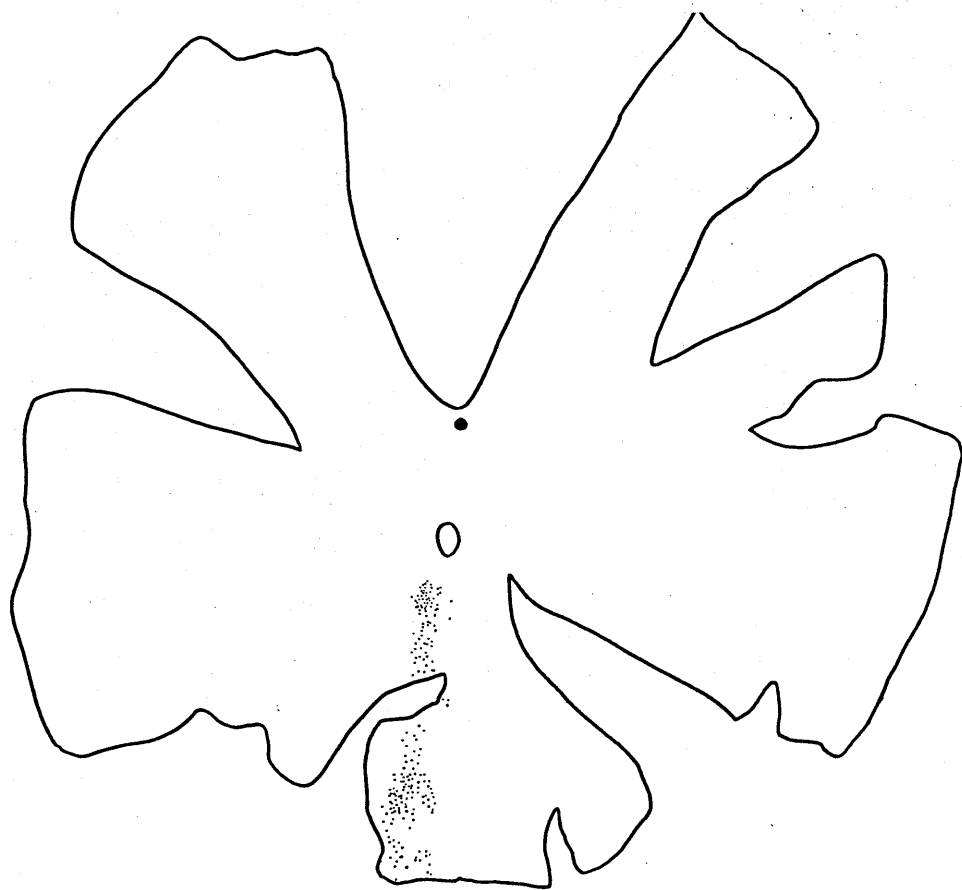
[ 90 ]







[90]

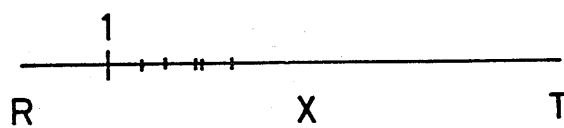
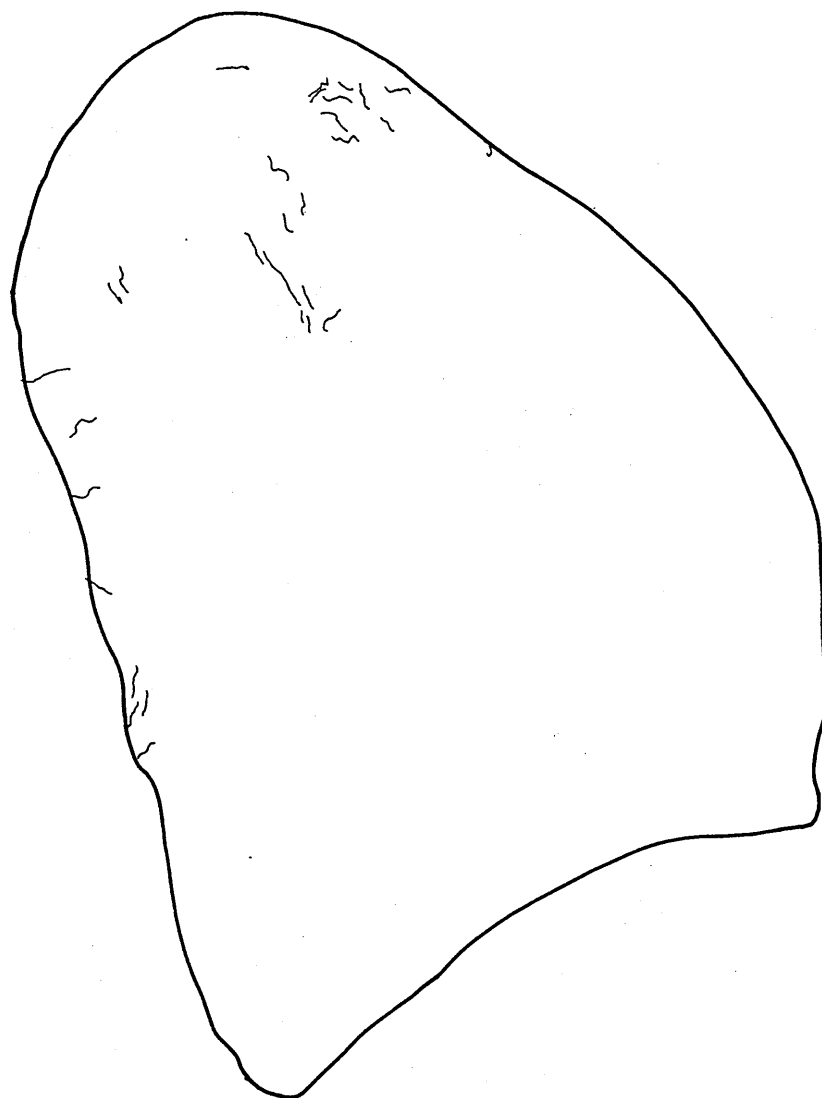


VENTRAL SECTOR

FIGURE 91

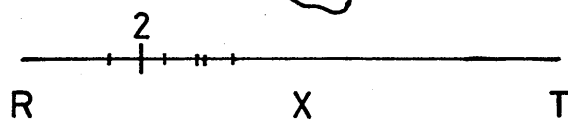
# REGENERATED PATHWAY : VENTRAL SECTOR

← Dorsal



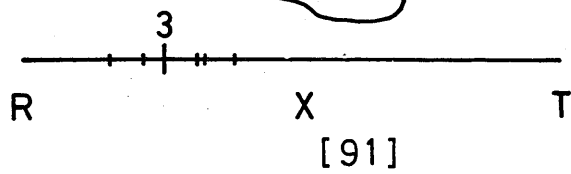
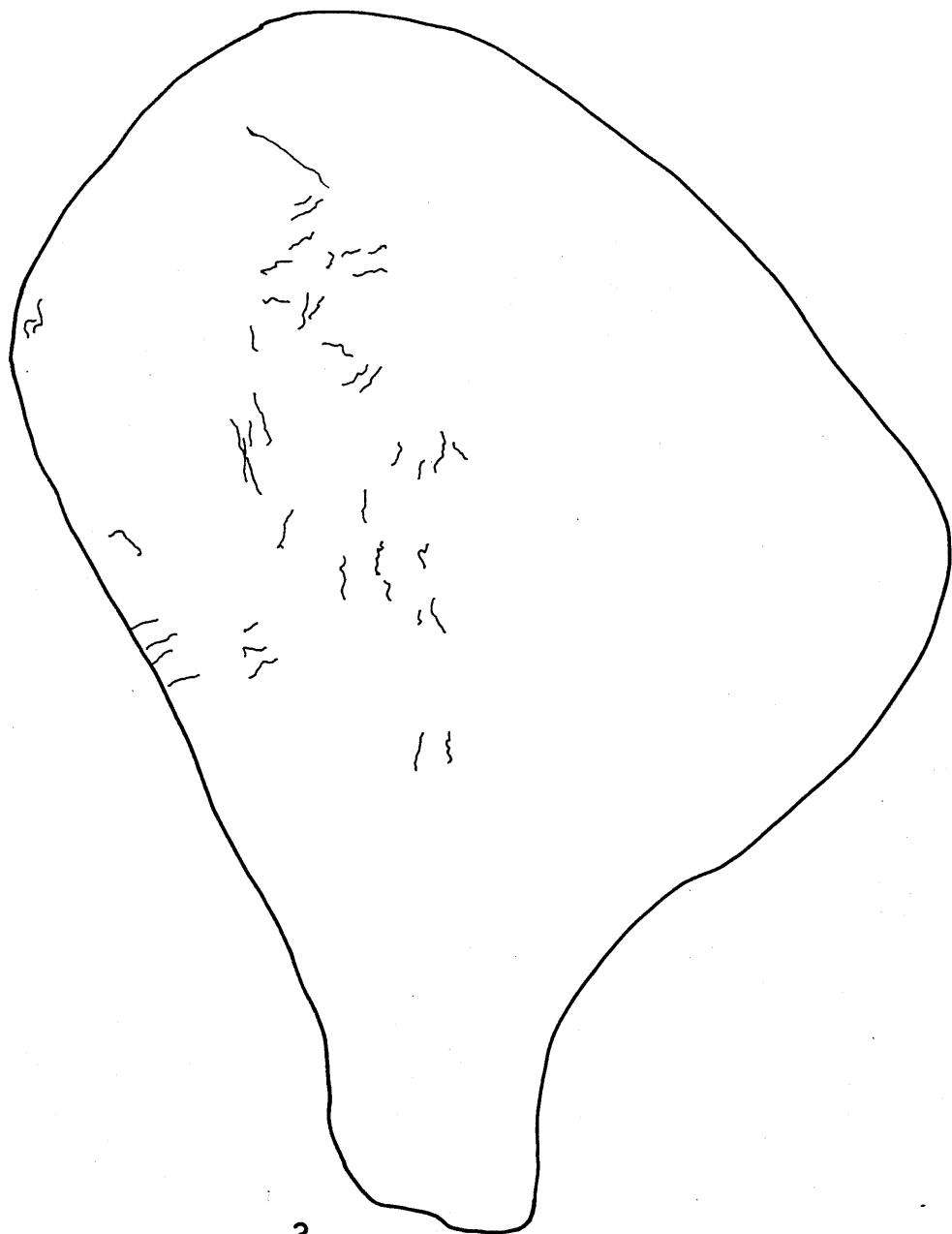
[91]

Dorsal  
←

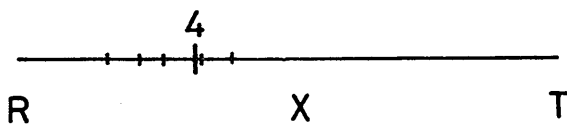


[91]

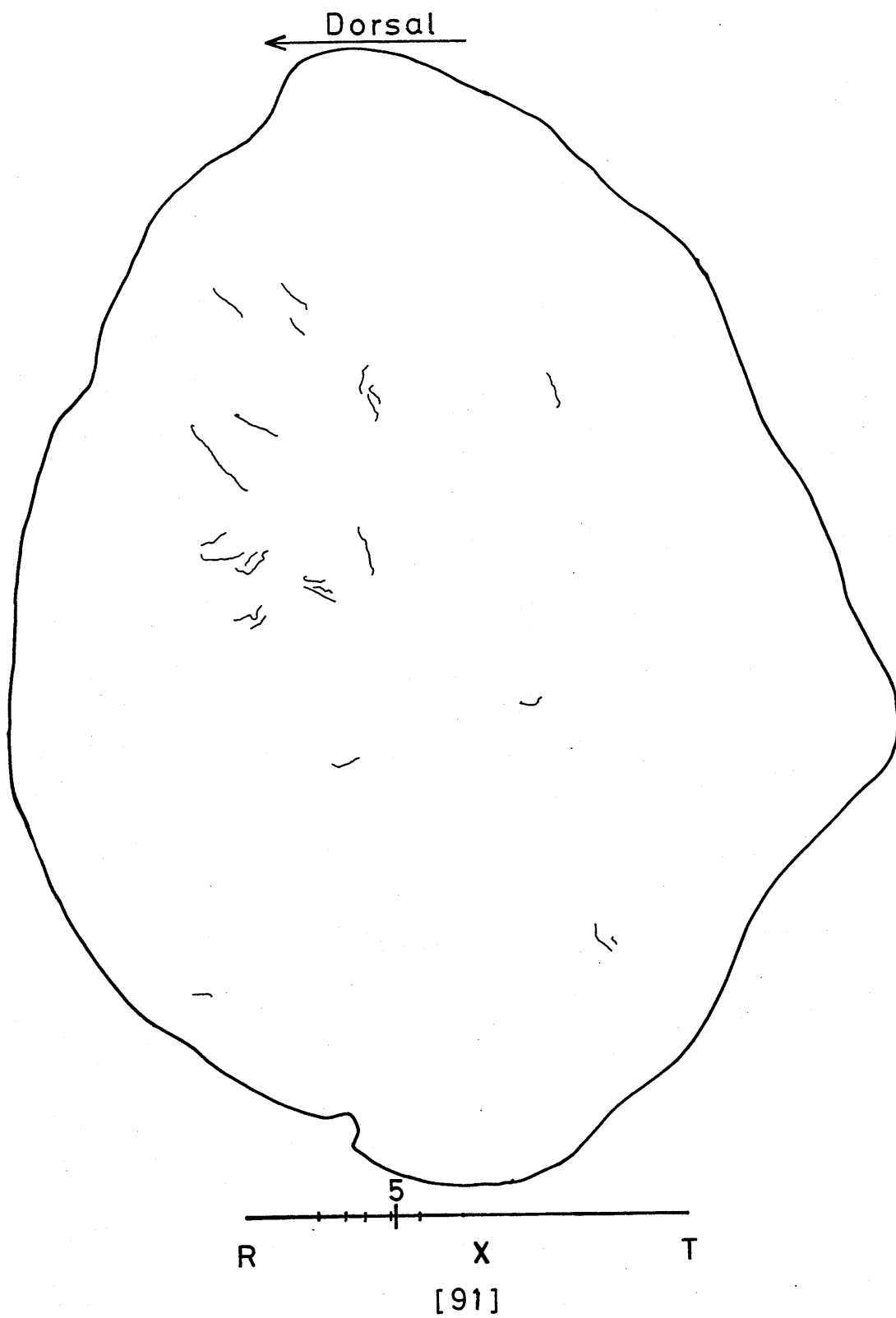
Dorsal  
←



Dorsal  
←



[91]



Dorsal  
←

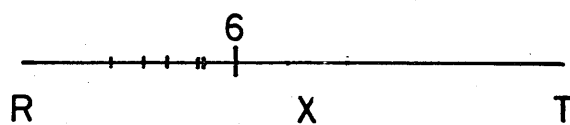
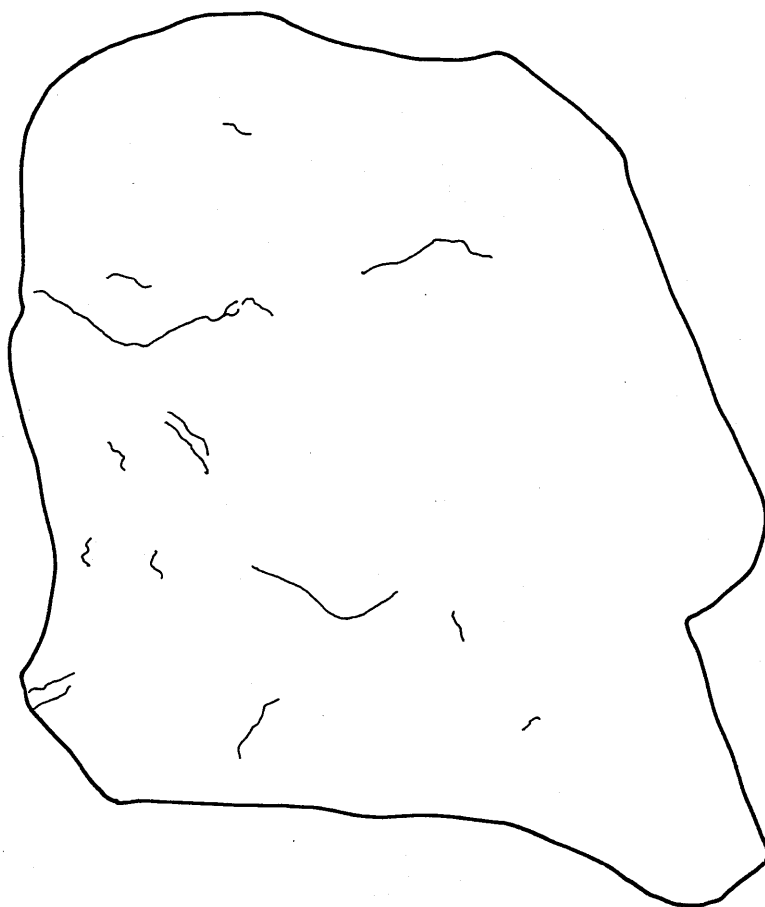




FIGURE 92

Visuotectal map: normal retinotectal projection after transection of axons from central retina (specimen shown in plate 10).

Open circles: no visually evoked responses.

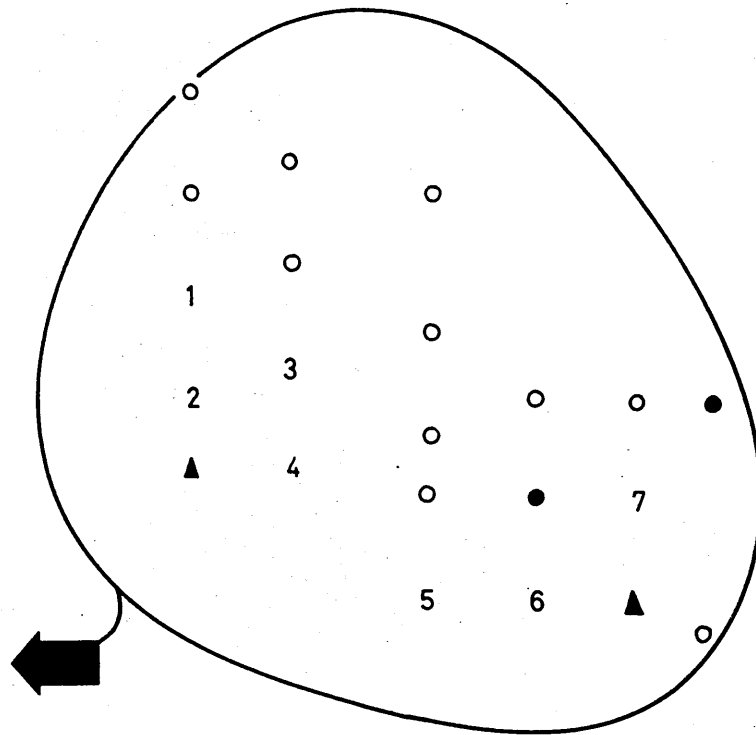
Filled circles: weak visually evoked responses, not localizable.

Numbers show corresponding tectal loci and visual field directions and confirm that the mapping of peripheral field is normal.

Solid triangles: visual field likely to have been obscured by apparatus; no information about retinal input.

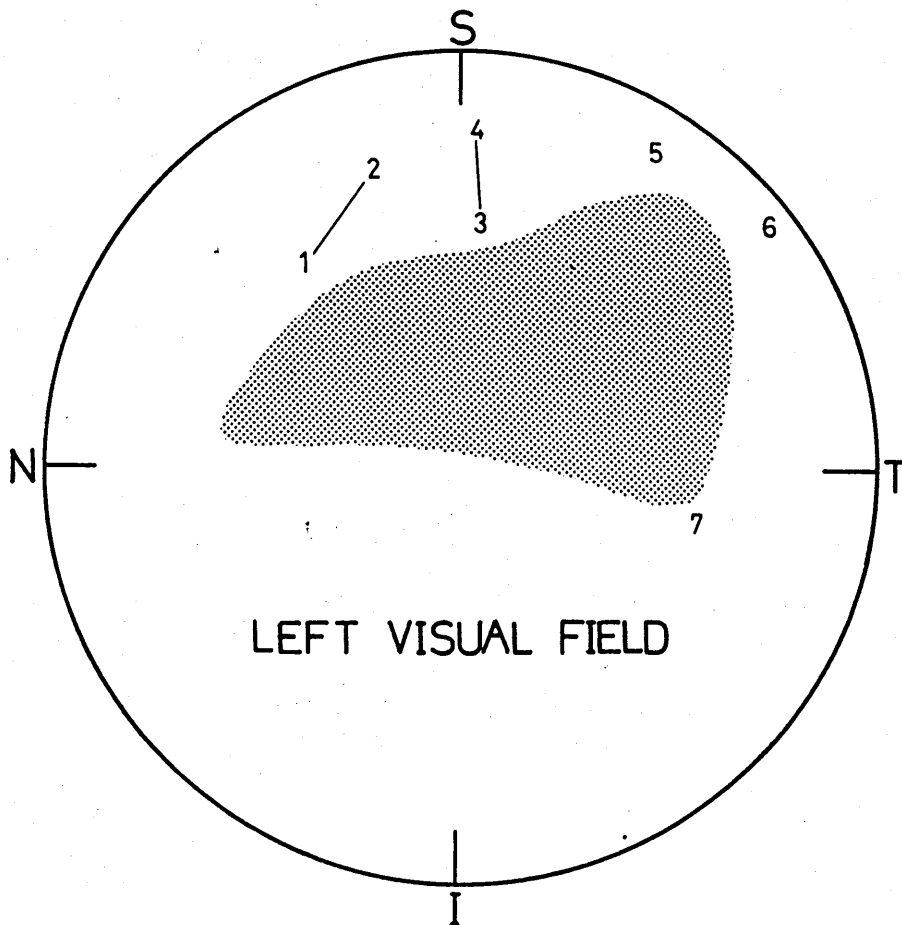
The solid arrow in the diagram of the tectum points rostrally in the midline.

N = nasal, S = superior, T = temporal, I = inferior.

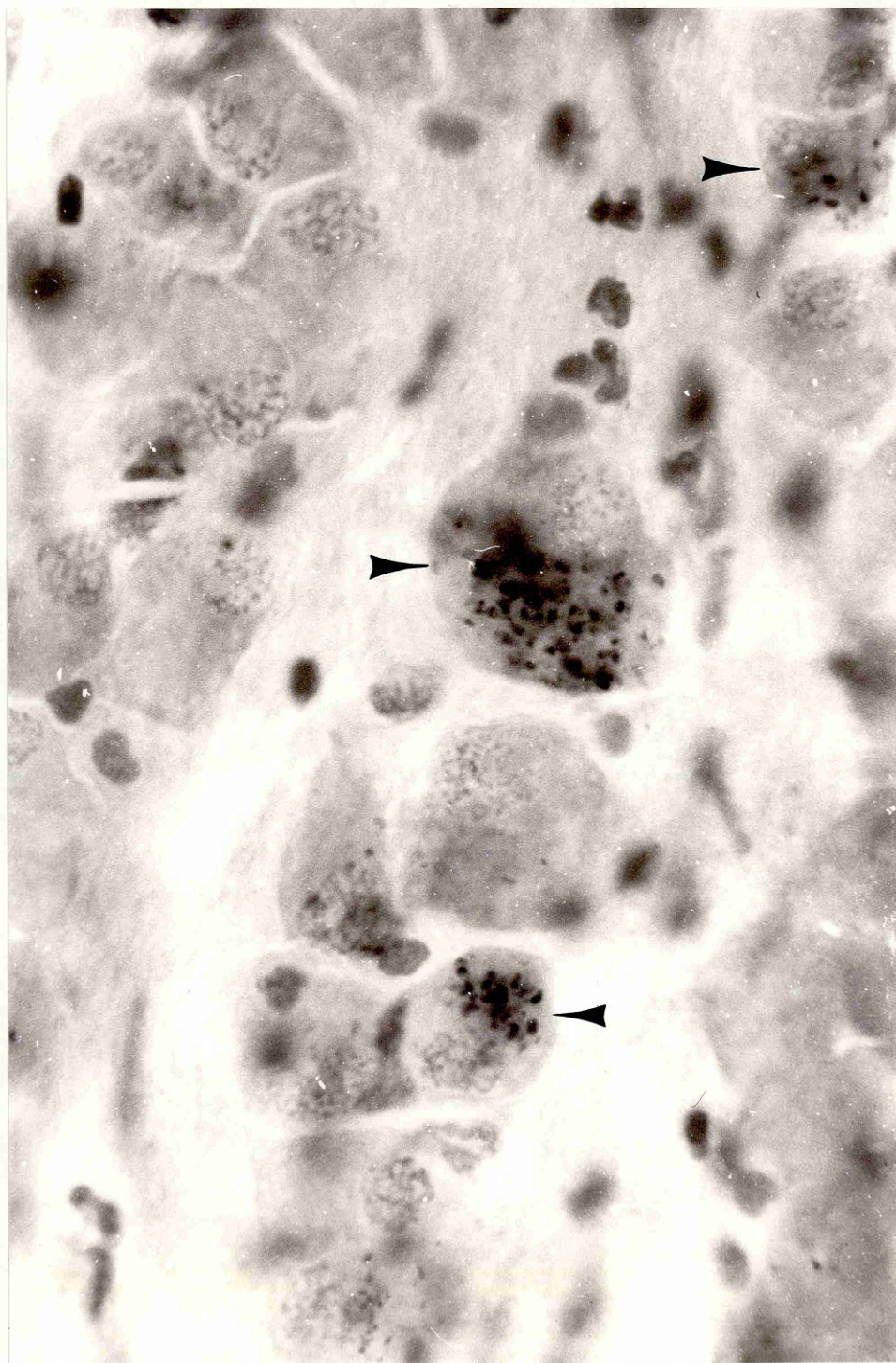


200  $\mu$ m

RIGHT TECTUM



LEFT VISUAL FIELD



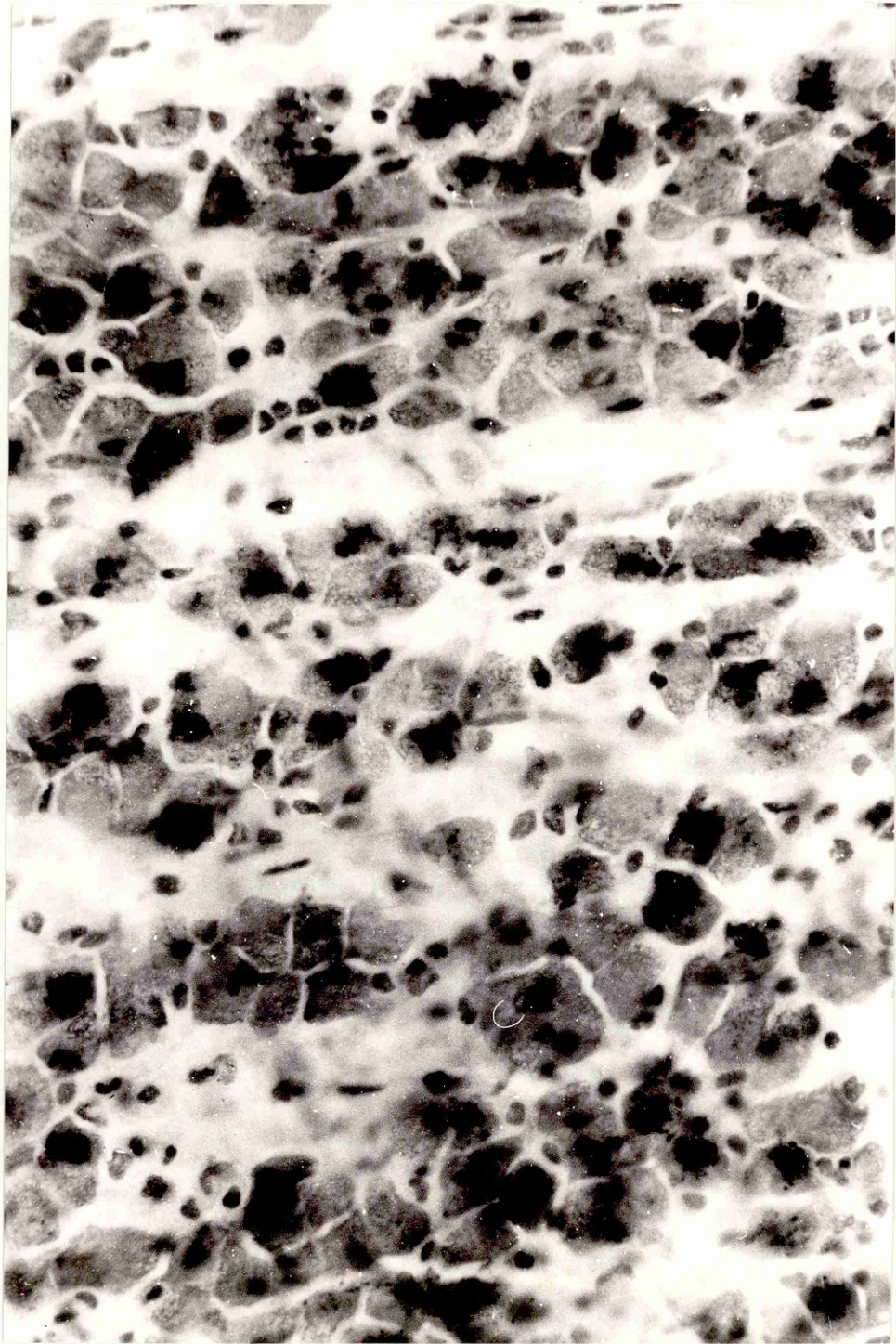
x1000

PLATE 1

HRP/DAB REACTION PRODUCT

() IN GANGLION CELLS





x400

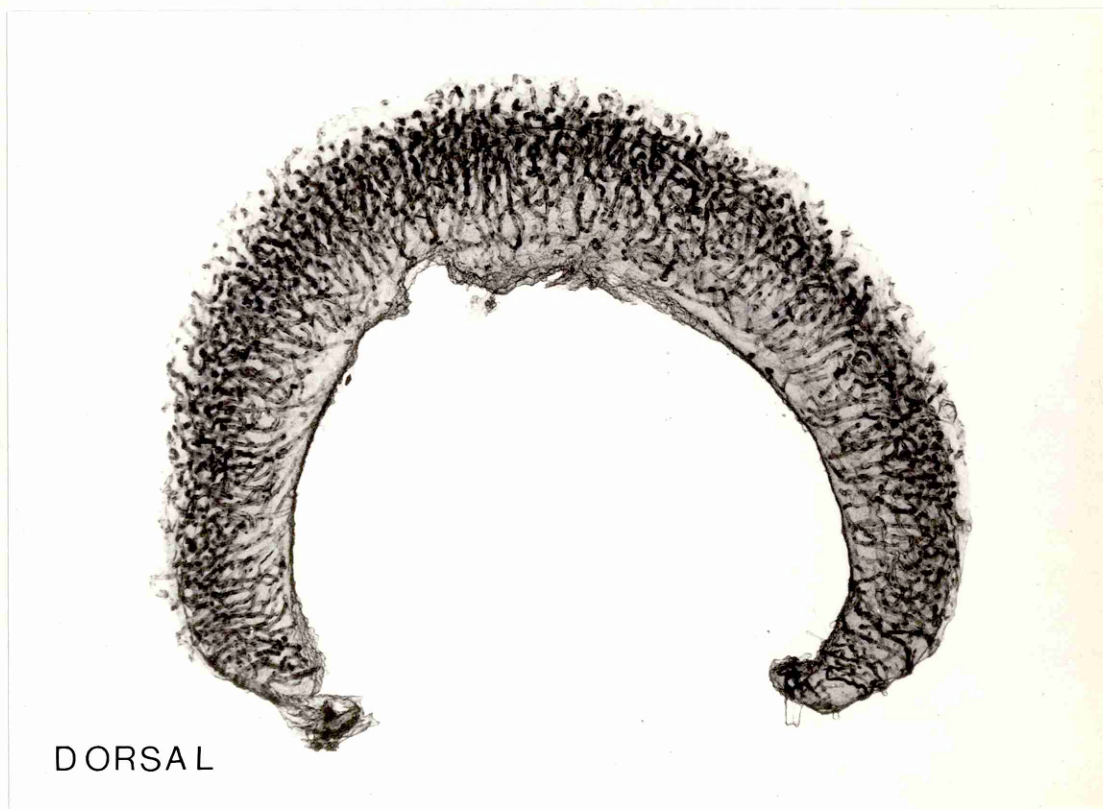
PLATE 2  
GANGLION CELLS HEAVILY  
LABELLED WITH HRP/DAB  
(BLACK DEPOSITS)





x63

PLATE 3: HORIZONTAL SECTION

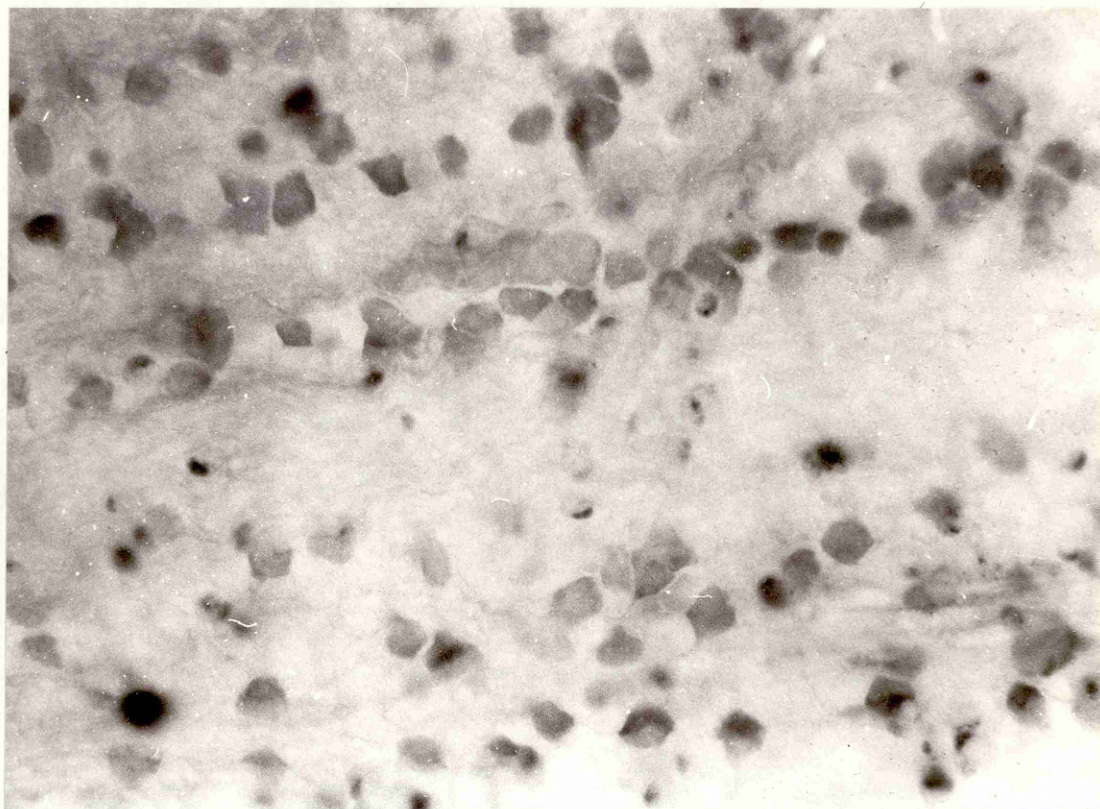


DORSAL

x40

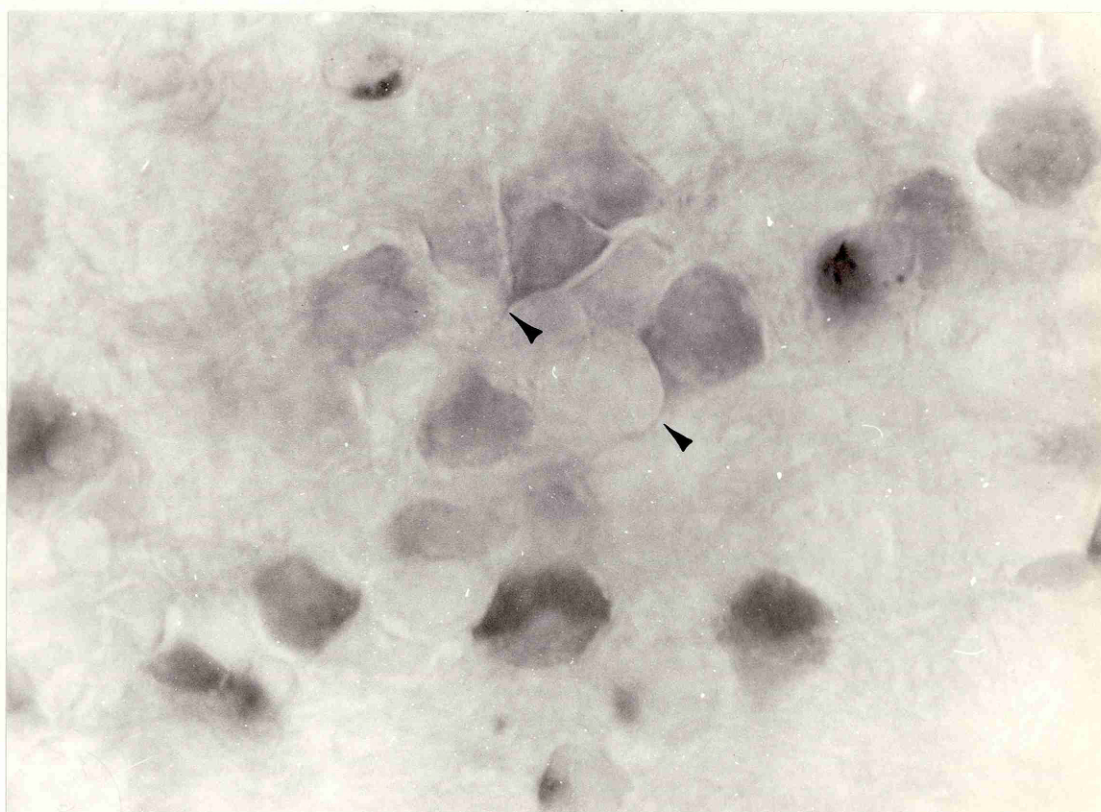
PLATE 4: CORONAL SECTION  
SHOWING CURVATURE OF TECTUM





x400

PLATE 5: HRP LABELLED GANGLION  
CELLS



x1000

PLATE 6: GANGLION CELLS WITH  
VISIBLE AXONS ▲





PLATE 7: LABELLED AXONS CONVERGING  
ON OPTIC NERVE HEAD (H) x160

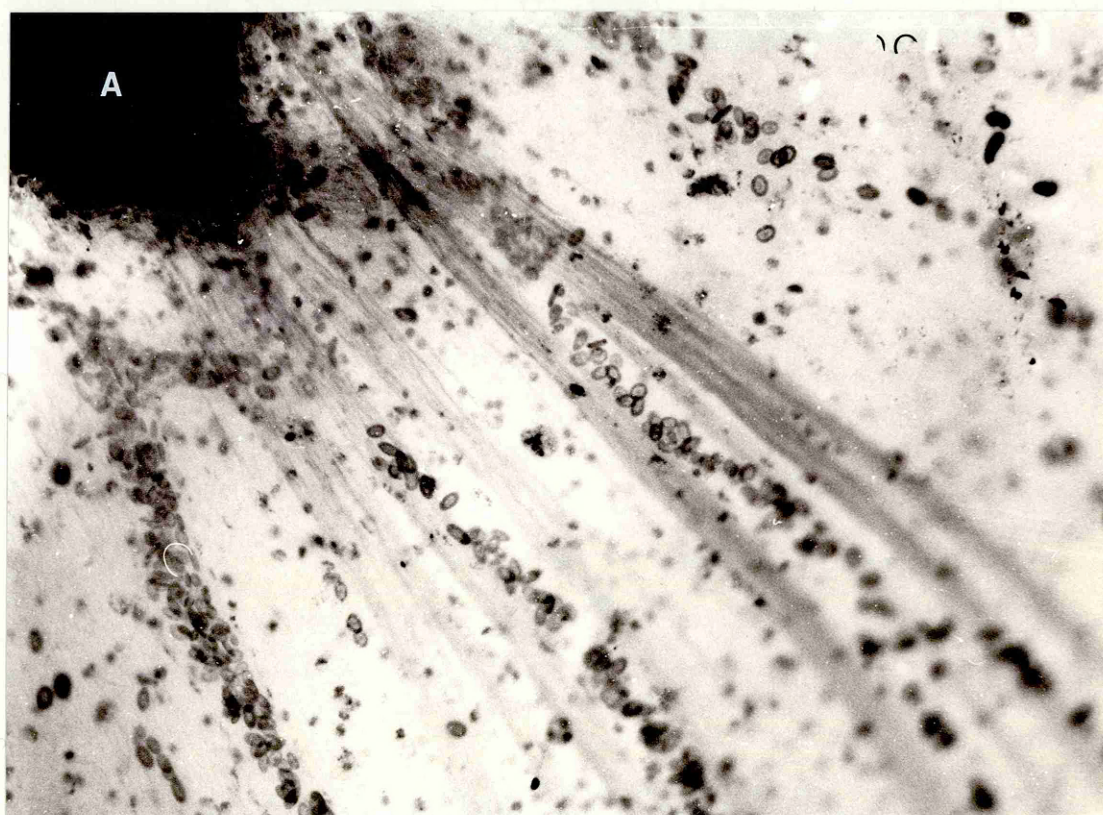


PLATE 8: LABELLED AXONS PERIPHERAL  
TO APPLICATION SITE (A) x160





x 128

PLATE 9 : HRP - LABELLED AXONS

(◆) IN OPTIC NERVE



PLATE 10

(Reproduced from Dawnay, 1981b, by kind permission of the author).

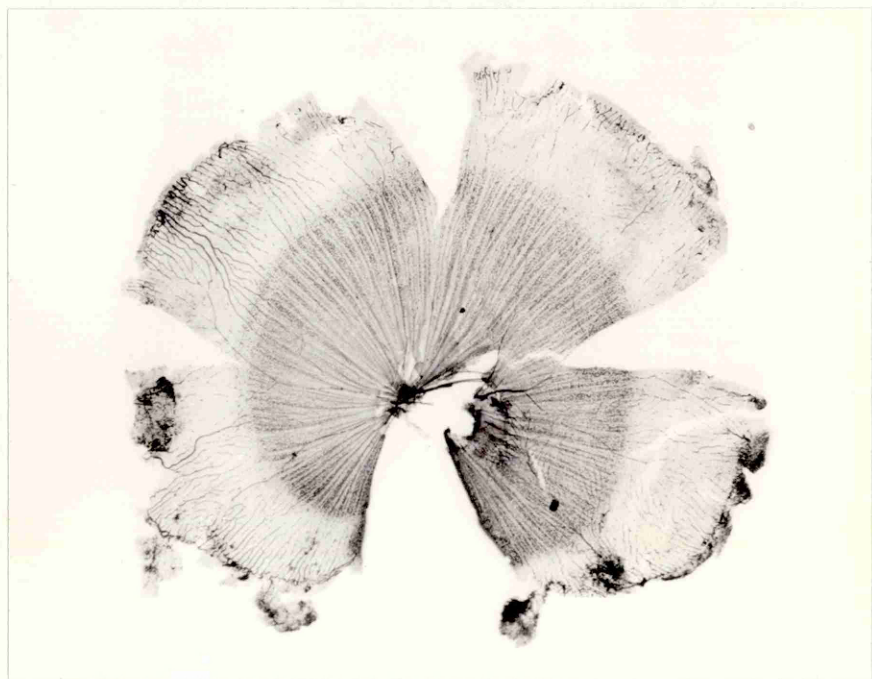
and PLATE 11

Normal goldfish retinae: central ganglion cells labelled with HRP applied to a cut through dorsal juxta-scleral optic nerve. See final appendix.



x10

PLATE 10



x10

PLATE 11